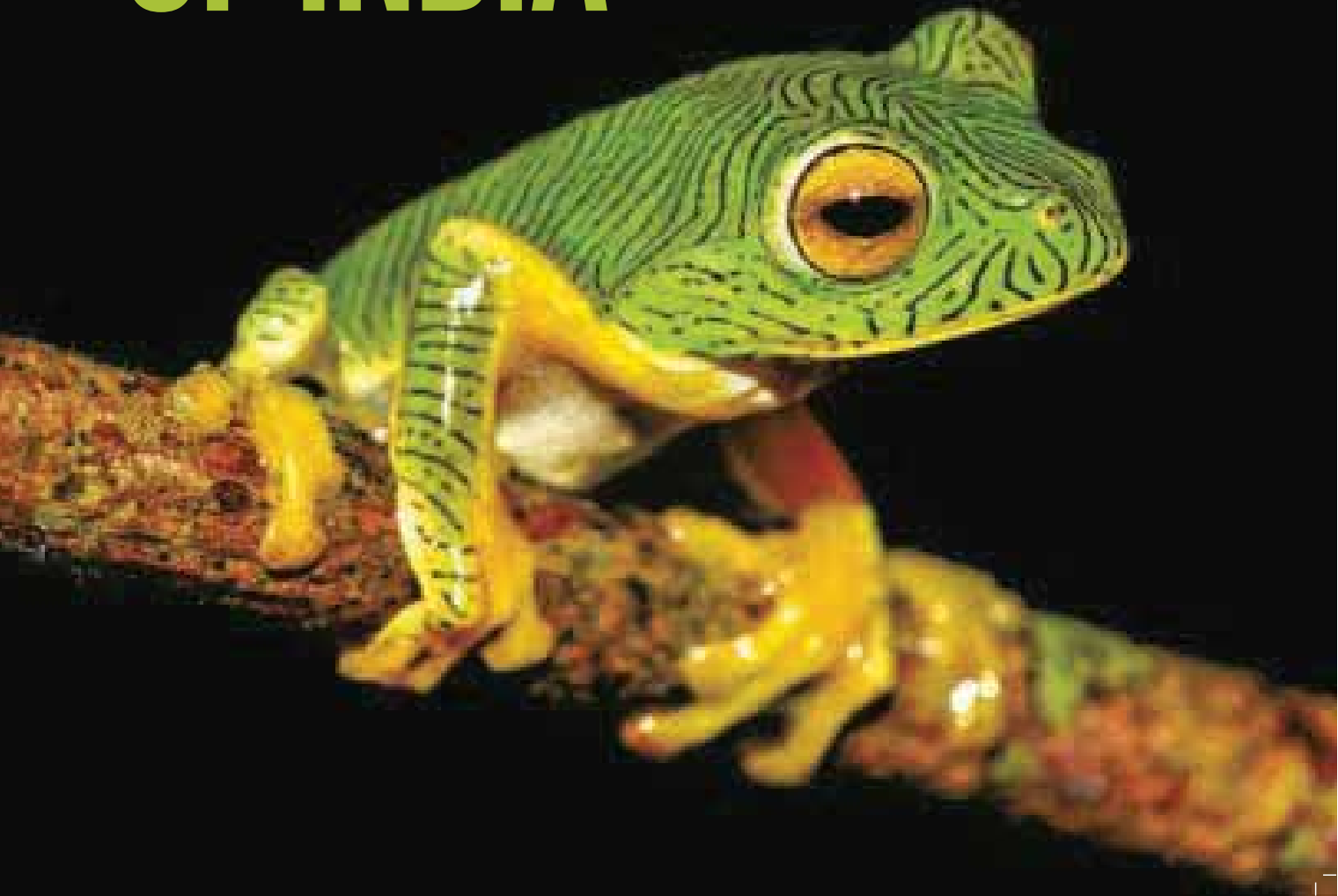


DIVERSITY & ECOLOGY OF AMPHIBIANS OF INDIA







DIVERSITY & ECOLOGY OF AMPHIBIANS OF INDIA

envis WILDLIFE &
PROTECTED
AREAS

ENVIS Bulletin

Diversity and Ecology of
Amphibians of India

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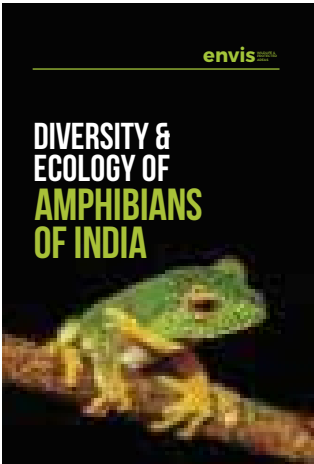
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write to:
Dr. Abhijit Das
abhijit@wii.gov.in

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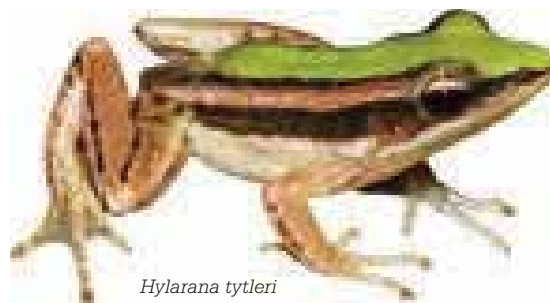
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Naitik Patel
Anant Pande**

Editorial Processing
Jyoti Prasad Nautiyal

Wildlife Institute of India
WII-ENVIS Centre
Chandrabani, Dehradun-248001, India Tel.:
+91135 2640114-115, 2646100
Fax.: +91135 2640117
Email: wii@envis.nic.in; envis@wii.gov.in
Website: <http://wiienvis.nic.in>;
<http://wii.gov.in/envis>



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The Environmental Information System (ENVIS) Centre at the Wildlife Institute of India, set up in September 1997, is part of the ENVIS setup of the Ministry of Environment, Forest and Climate Change, Government of India. It deals with general matters concerning 'wildlife' and specifically those related to 'Protected Area'.

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Rhacophorus suffry

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Amphibians, as predators of insect pests in agricultural and forest ecosystems, play a key role in regulating ecosystem processes and constitute a significant biomass, often exceeding that of all other vertebrates. They form an important link in the ecosystem as both predator and prey, contribute to environmental heterogeneity and foster important symbiotic associations with an array of organisms. In spite of their ecological significance, amphibians are often typically excluded from habitat management programmes and environmental impact assessments. Prioritizing amphibian conservation is also hindered by limited knowledge of their diversity, abundance and biological characteristics. Given that many species are habitat specialists, understanding their ecology and distribution can be used in monitoring habitat quality and integrity, and as indicators of unique habitats such as torrent streams, swampy habitats and forest floor. Quantitative data on their populations and habitats can potentially be used for constructing predictive models for monitoring of the target taxa and their habitats.

I am pleased to note a surge in amphibian research in our country in the past two decades. A staggering number of new species have been added in last 15 years or so making the total species count as 417. Ease in doing field work in remote habitats and use of molecular techniques in taxonomy have helped herpetologists to unearth many rare and cryptic species. Most of these discoveries have been made from two globally recognized biodiversity hotspots in India viz. Western Ghats and Northeast India. Amphibians in Western Ghats show remarkable endemism and thus can be used as model organisms to delineate and date biogeography of the peninsular India. Amphibian fauna of Northeast India on the other hand are largely of Indo-Chinese and Indo-Malayan origin and represent gateway characteristics for faunal distribution. Explorative and inventory studies need to continue, as special habitats such as evergreen forest canopy, remote forests of northeast India and Nicobar Islands have the potential to provide new species. It is now important to move ahead and start collecting biological and population level data for individual species, which would also help us to prioritize conservation of amphibians.

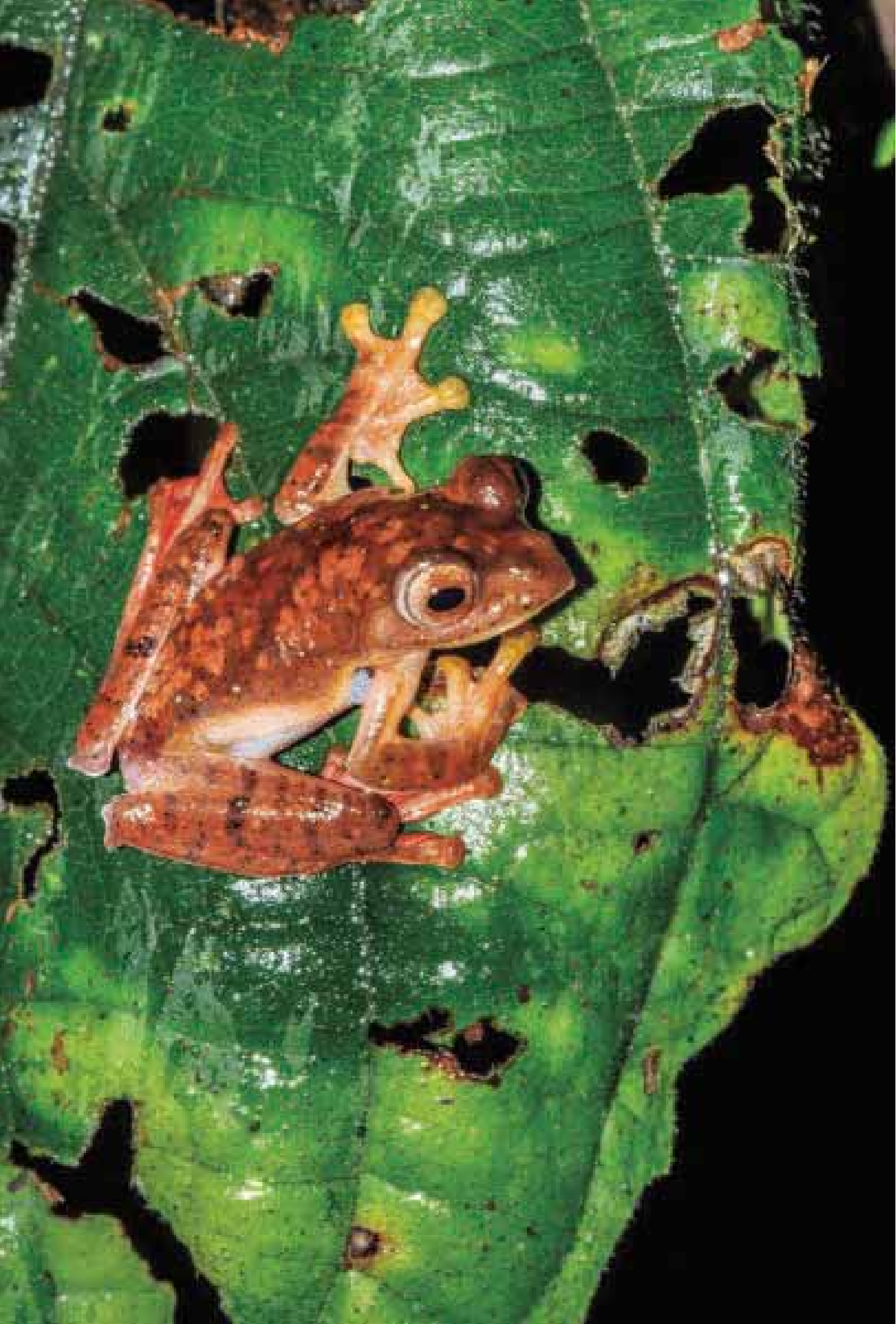
Amphibians are the most threatened group of vertebrates globally. Their biological characteristics of dual mode of life, anamniotic eggs, porous skin, low dispersal ability and habitat specificity make them particularly sensitive to habitat loss, pollution, and fragmentation. Realizing the fact that a significant diversity hitherto remains to be documented, we are facing the tragedy of 'nameless' extinction. At the same time we need to gather information on population and ecological characteristics. The declining population paradigm is the greatest conservation challenge. Future survival of the amphibians will depend largely on the decisions that we as humans would take now. However, looking to the future, the fate of amphibians seems optimistic considering the public attention they are now able to garner and the information that continues to get generated on them as reflected in this bulletin.

This issue of ENVIS takes a beyond taxonomy approach and delves into poorly known ecological and biological aspects of amphibian research in India. The bulletin includes an inspiring foreword by veteran herpetologist Professor S. K. Dutta and original research and review papers by leading amphibian researchers of the country. It is hoped that this publication will help generate enthusiasm among the readers to move ahead in amphibian research and conservation in the days to come!

I look forward to receiving your valuable feedback on this issue.

1st September 2017

V.B. Mathur
Director, WII
& Project Coordinator,
WII- ENVIS Centre





Foreword

Indian amphibian research was attributed to research at Colleges and Universities during and few years after British era. Basically, teachers of many Colleges and Universities use to conduct various biological research (Cytology & genetics, developmental biology, biochemistry, physiology, parasitology, osteology, comparative anatomy, morphology, toxicology, myology etc.) using amphibians (frogs, toads, salamanders & caecilians) as research animals. The most common species of frogs and toads used for such research were *Bufo melanostictus* (= *Duttaphrynus melanostictus*) *Rana tigrina* (= *Hoplobatrachus tigerinus*) and *Rana hexadactyla* (*Euphyctis hexadactyla*). Also, before independence of India, Zoological Survey of India, Bombay Natural History Society and a handful of college and university teachers also conducted taxonomic research. However, there has been a halt to such biological research using amphibians as a model and this is because of ban on dissection of animals in the laboratories and also strict enforcement of Wildlife Protection Act (1972).

In spite of all the odds for laboratory research, there has been much emphasis on field research dealing with ecology, natural history, breeding biology, bioacoustics and taxonomy. A new dimension has been added to taxonomic research of amphibians of India due to advent of molecular research dealing with Barcoding and DNA sequencing. Instead of pure morphology, there has been amalgamation of morphology and molecular research to establish novelties of fauna and new species are being added to the existing number of species. The first publication providing systematic descriptions of the amphibians of "British India" (consisting of India, Sri Lanka, Nepal, Pakistan, Bangladesh and Myanmar) was "The Reptiles of British India" by Albert Günther during 1864; in all 39 species of amphibians were included in this account. Subsequently, George Boulenger's 1890 Catalogue (Fauna of British India) included 130 species of amphibians to be found in India. During 1986, I and Robert F. Inger (Field Museum of Natural History Chicago, USA) made an initial attempt to compile the species list of all the amphibians. Subsequently, my book "Amphibians of India and Sri Lanka: Checklist and Bibliography" contained a detailed distribution record and also the most recent taxonomic treatment of all the species known till 1997. Now the total number of species stands at about more than 415. This indicates the interest and tenacity of Indian amphibian researchers in discovering cryptic species populations.

The maximum numbers of new species discoveries have been from the Western Ghats and the Northeast India from where endemic families, genera and species are being described continuously. This is due to well trained experts in the field of herpetology in India. More than 200 researchers have been trained during the last 10 years through funding from the Department of Science & Technology, Govt. of India in the form of School in Herpetology and three such training programs have been organized at Wildlife Institute of India (WII), Dehradun. Hence, appropriately, the WII is bringing out the first compilation on various aspects of biological studies of Indian amphibians. Interestingly enough, all the authors of articles included in this publication have been the part of "School in Herpetology" (either faculty or trained researcher from various parts of the country). This reflects towards a great future for herpetological research in the country. As we know, amphibians are yet to be considered under various management and conservation programs formulated by the State Forest Departments. Though they play a major role in the ecosystem (aquatic, terrestrial, arboreal and fossorial etc.) and many of them are bio-indicator species, there has been much neglect to assess their biological needs. The present publication includes most of the articles on various biological aspects of Indian amphibians and hopefully, this could be used by policy makers, scientific community and managers towards formulation of Action Plan for future conservation of amphibians in the country.





Preface

Class amphibians have descended from a diverse group of tetrapod vertebrates that first appeared during Devonian Period, about 400 million years ago. Characters that make amphibians different from other vertebrates are a three chambered heart, a reproductive cycle that includes larval stages called tadpoles and a body covered with naked moist skin (except in some species of caecilians, which show tiny scales). The larval stages respire with the aid of gills and skin, while after metamorphosis, amphibians breathe with the help of their lungs and/or skin.

The most widely recognized group among the amphibians is the Anura (tailless amphibians) that include frogs and toads. They have tarsal bone, hind limbs larger than the forelimbs, large eyes, and remarkable shortened ribs and trunk. Anurans are widespread in tropical and temperate region. Out of 7707 known amphibian species globally so far, 88% are anurans.

The Gymnophiona (caecilians or limbless amphibians) are globally represented by 206 species. They have an elongated body having primary and secondary annular grooves, vestigial eyes and a small tail. Fertilization is internal, females may be either oviparous (egg-laying) or viviparous (give birth to fully developed young). Egg-laying caecilians are known to guard their eggs. Caecilians are tropical in distribution and their ecology is poorly known. India is one of the center of origin for caecilian diversity and endemism. Chapter 04 reviews current knowledge on caecilian diversity and distribution in poorly known Northeast region of India.

The third group, Urodela or Caudata (salamanders and newts) are represented by 704 species globally. They are characterized by a well-developed fore and hind limbs (except Sirenidae, hindlimbs are absent), distinct tail and neck, reduced eyes and absence of tympanum. Caudata are poorly represented in India and so far two species are known from Himalayan and adjoining Northeast region. We get a glimpse of the distribution localities of the newly described Himalayan salamander (*Tylototriton himalayanus*) from chapter 09.

Currently, 417 species of amphibians are known from India. Interestingly 43% of this diversity is described in just last 15 years. Chapter 02, Chapter 05 and Chapter 07 review the issue of new species discovery and natural history studies from Odisha, Andaman & Nicobar Islands and Maharashtra respectively. Diversity and endemism are exceptionally high in Western Ghats mountain chain with unique families such as Nasikabatrachidae, Ranixalidae, Micrixalidae and genera *Nyctibatrachus*, *Uraeotyphlus*, *Gegeneophis*. Northeast India, on the other hand represents fauna typically of Indo-Chinese and Indo-Malayan origin. Unique family of this region is Chikilidae. Amphibian species richness progressively decreases in central Indian landscapes and westwards, with increasing aridity.

Geographical distribution of amphibian in the heterogeneous environmental setup of India is thus an intriguing component of scientific study. Understanding factors governing the geographical distribution of species remains the central focus in community ecology (Pianka, 1966a; MacArthur, 1972). Elevation distribution pattern although initiated long back (Grinnell and Storer, 1924), has got scanty attention in India (Naniwadekar and Vasudevan 2007, Chettri, 2007, Acharya et al, 2011, Raman et al. 2005). Chapter 06 reviews elevation distribution pattern of amphibians in the eastern Himalayan region.

Most herpetologists would agree that the tadpole stage is just as crucial as the adult stage, and may play a decisive role in distribution and speciation processes, particularly in tropical assemblages. Dual mode of life of anurans imposes different selective regimes during aquatic larval stage, the metamorphic stage, and the terrestrial post-metamorphic stage (Wilbur & Collins 1973, Wassersug 1997, Rose 2005). The study of tadpoles offers a tremendous potential for addressing ecological and evolutionary questions. New species can be discovered by the collection and identification of their larvae, when adults are rare or cryptic (Inger, 1985). However, lack of state-of-the-art tadpole descriptions, inventory catalogues, and reliable determination keys is a major impediment to inventory and ecological research on the larval amphibian (Hass and Das, 2011). Chapter 08 thus addresses the issue with a robust morphological key for identification of larval tadpoles.

Acoustic characteristics of anurans are species-specific and can be used for their identification, description of cryptic species, understanding phylogenetic relationships among species (Modak et al, 2016). In some species, the quality and structure of male calls influence the outcomes of male-male contests and female choice (Bee, 1999; Yu & Zheng, 2009). Females are known to prefer males with calls of lower fundamental frequency or that can sustain calling longer than their competitors (Howard & Young, 1998; Ryan & Drewes, 1990). Call surveys are a widely used and accepted monitoring technique for predicting anuran calling activity (Kirlin et al, 2006). Chapter 03 describes identification of a cryptic species based on call characteristics.

Anamniotic eggs of amphibians require special care. Oviposition sites are crucial to ensure egg protection from predator, parasites, dehydration and thermal stress. Selecting a potential oviposition site by adult females thus determines the survival of the offspring and reproductive success (Wells 1977). An appropriate choice of oviposition site is especially critical for oviparous animals that lack parental care (Murphy 2003). Oviposition sites are species specific and knowing them are important from research and monitoring purpose. Chapter 10 describes oviposition sites of frogs from Northeast India.

Studies on the reproductive behavior of amphibian species in India has been scanty and data on breeding behavior are available for roughly 7-8 percent of the total amphibian species of the country. Studies on the breeding biology of amphibians is of crucial importance for the successful conservation the species along with their habitats (Gaitonde et al. 2016), and more so for endemic and poorly known species. In this ENVIS bulletin, two articles are dedicated to the detailed breeding behavior of two anuran species (Chapter 12 and 13).

Dietary information is essential to understand amphibian life history, population fluctuations, and the impact of habitat change. Studies on diets are essential for assessments of energy flow and food webs in ecological communities. Dietary specialization is often associated with morphological, physiological, and behavioral characteristics and is pivotal for successful development of conservation strategies on species level and the understanding of ecosystem function. Unfortunately, this kind of information is not available for the vast majority of taxa and is often incomplete. Chapter 15 reveals dietary patterns and niche overlap in five sympatric anuran species assemblages in Uttarakhand region.

Amphibians help us in numerous ways. Frogs are effective controller of agricultural insect pests. In each hectare of paddy, frogs save tons of rice by consuming insect pests. As bioindicators of environmental health, many amphibian species are used to detect high nutrient load in waterbodies, in addition to radioactive contamination, thermal and chemical pollution. Tadpoles pick up metals from the surface of sediments, making them potential indicators of contaminated environments. Skin toxins from poisonous frogs are potential sources of new drugs and many species are used in biomedical research, including in transplant immunology. Amphibians are also used as model organism for regeneration studies and chapter 11 is a contribution towards understanding effect of retinoid in amphibian tail regeneration.

Amphibians in India are facing serious threat to their survival. Habitat loss and fragmentation for development activities and monoculture plantation are major threats to forest species. Harvesting large growing dicoglossid frogs for meat threatens adult breeding population. Agricultural and industrial chemicals are detrimental to amphibian populations. Chapter 14 shows the impact of such pollution in few perianthropic species. Similarly, linear infrastructure projects, especially roads, are a major cause of amphibian population decline and extinction worldwide (Forman et al. 2003, Puky et al. 2007, Fahrig et al. 1995). In India, direct and indirect impacts of roads on amphibians are poorly known. With the increase in inevitable development, mitigation measures are the cornerstone of conservation. Chapter 18 is an effort towards reviewing the current knowledge on impacts of linear infrastructures on amphibians with possible mitigation measures.

Prioritizing amphibian conservation is of paramount importance in the current scenario. Can amphibians be used as flagship species for conservation? Chapter 17 is a first of its kind approach towards identifying potential flagship Indian amphibian species. This approach might help in building appreciation towards this lesser known vertebrate group and improve local support for on-ground conservation.

I hope this ENVIS bulletin on Diversity and Ecology of Amphibians of India will garner the interest of research community and would help generate an integrated approach towards conservation of Indian amphibians.

Abhijit Das

Wildlife Institute of India,
Chandrabani, Dehradun, Uttarakhand
Email: abhijit@wii.gov.in

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Namdapha Tiger Reserve, Arunachal Pradesh
Photo Credit: Monaj V. Nair

SECTION

I

DIVERSITY AND DISTRIBUTION

Some Early Indian Amphibian Illustrations and Their Artists

Abstract

Amphibian researchers will benefit from examination of early works, including books and papers by Nineteenth Century zoologists, most of which are illustrated with black-and-white or hand-coloured lithographic or engraved images of the subject. This essay deals with selected works from the period, and discusses such artwork and their artists and authors.

Introduction

The history of India's amphibian studies dates back centuries, and feature workers, both western and Indian (Adler 2014; Das 2004). In this essay, we discuss images and artists of sketches and paintings of Indian amphibians in selected early (19th Century) works of natural history and taxonomy. These sometimes illustrated works resulted from the efforts of Europeans, either long-time residents of India or those based in European (especially in England or France) museums. The artworks were produced by uncredited local artists (as in 'Illustrations of Indian Zoology', 1800-1835) or by leading painters and lithographers of the day (such as those in the catalogues of the British Museum or in scientific periodicals of the time).

Gray's (1830-1835) Illustrations of Indian Zoology

Little of the Indian fauna was known to the west prior to the turn of the Nineteenth Century. The administrators and soldiers of the English establishment frequently turned to nature, many of whom made lasting contributions to the Subcontinent's natural history.

Thomas Hardwicke (1756-1835; Fig. 1) joined the Bengal Artillery of the East India Company as Lieutenant Fireworker in 1778,



Key words

India, amphibians, literature, bibliography, illustrations, art.

Fig. 1.
Major-General Thomas Hardwicke (1756-1835).
Source: Gray (1830).

rising to the rank of Major-General. However, he is arguably better known as a collector of natural history specimens, especially vertebrate animals. He also collected coloured sketches of plants and animals, which comprised 32 folio volumes, that included over 2,000 drawings. They were based on specimens collected/observed by local artists in Hardwicke's pay around places he was posted, chiefly, Bengal and the United

Indraneil Das¹ & Sushil K. Dutta²

¹Institute of Biodiversity and Environmental Conservation
Universiti Malaysia Sarawak, 94300, Kota Samarahan
Sarawak, Malaysia

²Indian Institute of Science, Bangalore 560 012, India
Corresponding author e-mail: idas@unimas.my

Boulenger's (1880-) British Museum Catalogues

Two prominent European museums started the tradition of printed catalogues of the world faunas in their collection. The tradition of printing museum specimen catalogues for the British Museum was started by John Gray, who amassed a million specimens during his employment at the institution (two earlier catalogues of this institution showing no trace in the Museum's minutes records; Sherborn 1926, 1934). The publisher of the British Museum catalogues, from the middle of 1800s, was Taylor and Francis, a company that survives to this day. The British Museum series includes the amphibian catalogues (1882a; 1882b), prepared by George Albert Boulenger (1858-1937). The proprietors of the company were Richard Taylor, who started the Philosophical Magazine, an early scientific journal and William Francis, a chemist, in 1852 (Brock & Meadows 1998). The catalogues were printed with a mass market in mind, the volumes containing black-and-white lithographed plates, executed by leading British artists and lithographers of the time.

Lithography (from the ancient Greek roots, lithos, for 'stone', and graphein, or 'to write') is a printing technique, whereby printing is from a piece of lithographic limestone, or metal plate (at present, polymer coating applied to plastic or metal plate) that can print both text and figures on paper. Lithographic prints were at the time hand-coloured, a laborious process achieved by semi-skilled artisans working in an assembly line. The lithographic prints of the catalogues were mostly produced by the Mintern Bros, described as Chromo-Lithographers and Printers, with an office in Bloomsbury Mansions, London (between 1870-1905). Other plates were executed by Edwin Wilson (1855-1915) and George Henry Ford (1809-1876). Little information can be found on Wilson, apart from that he was a lithographer and an

entomologist. Ford, who worked for John Gray (see Gunther 1972, for a biography), is relatively better known, and was a South African natural history illustrator, most famous for his plates of Smith's "Illustrations of the Zoology of South Africa" (1838-1847).

Figs. 5-6 show images from Boulenger's catalogues of Toad and caecilians, both lithographed by the Mintern Brothers.

Fig. 5. Lithograph from Boulenger's (1882a: Plate XX) Catalogue by the Mintern Brothers, showing *Duttaphrynus himalayanus* (Günther, 1864: as *Bufo himalayanus*).



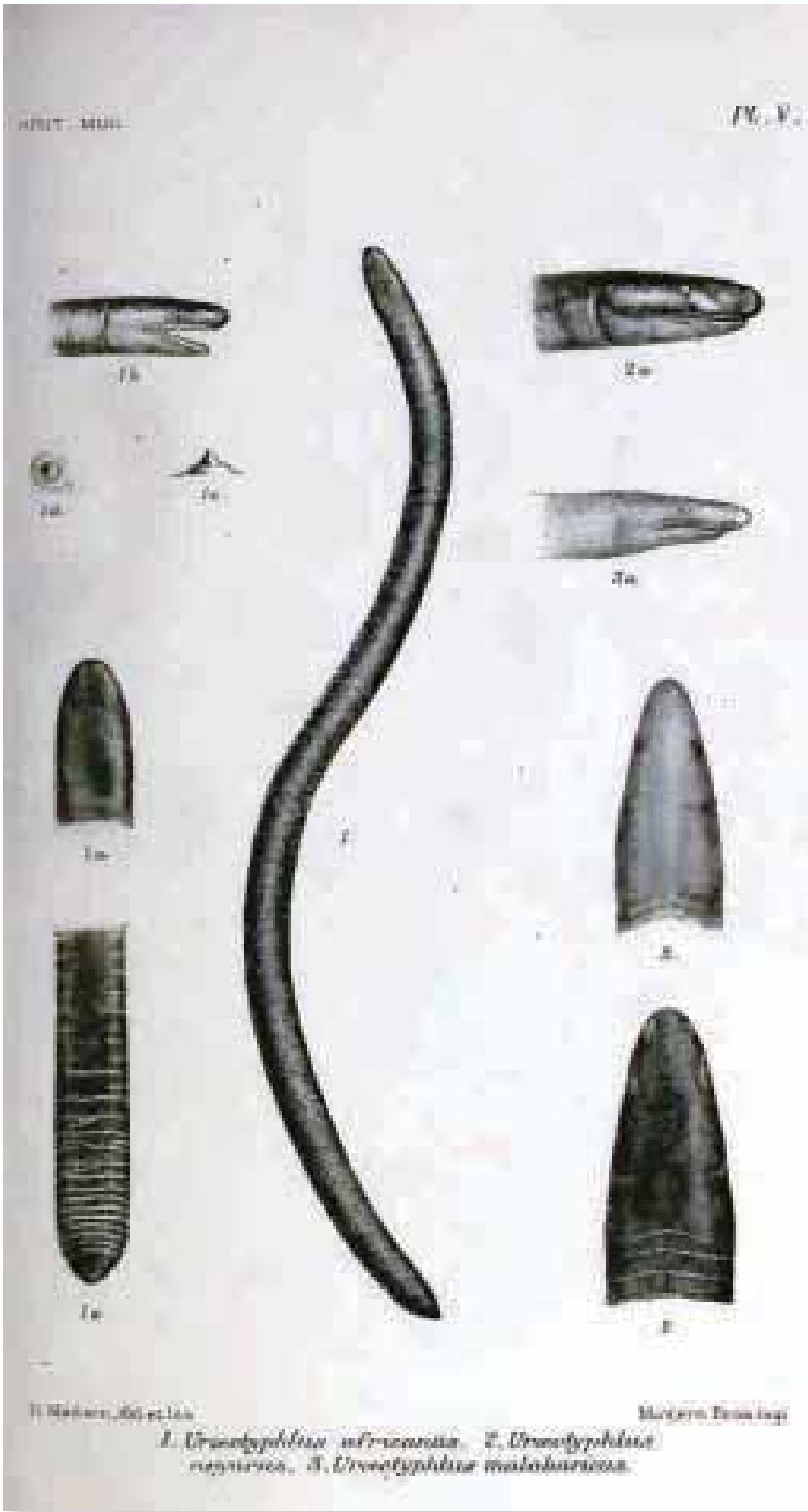


Fig. 6. Lithograph from Boulenger's (1882b: Plate V) Catalogue by the Minter Brothers, showing two Indian species, *Uraeotyphlus oxyurus* (Duméril & Bibron, 1841) and *U. malabaricus* (Beddome, 1870), as well as the African *Geotrypetes seraphini* (Duméril, 1859: as *U. africanus*).

Daudin (1802)'s Histoire Naturelle des Rainettes

The French scientist François-Marie Daudin (1776-1803) suffered from paralysis of his legs from childhood, and died short of his 27th birthday. While Daudin was better known for his eight volume French encyclopaedia of reptiles, published between (1801-1802) and described over 500 species), he also authored a significant work of 108 pages on frog taxonomy, entitled "Histoire Naturelle des Rainettes, des Grenouilles et des Crapauds" (Daudin 1802; Fig. 7), with a large number of beautifully reproduced lithographs of frogs (Figs. 8-9). The work was published in 1803 (Adler 2014). Daudin's most well-known Indian frog species description is that of *Rana tigrina* (current name: *Hoplobatrachus tigerinus*).



Fig. 7. Title page of Daudin's (1802) unicolored quarto version of *Histoire Naturelle des Rainettes, des Grenouilles et des Crapauds*

Fig. 9. Illustration from Daudin (1802: Pl. XXXV; p.96) of *Duttaphrynus melanostictus* (Schneider, 1799: as *Bufo bengalensis*); top. On bottom is the African *Brevicpes gibbosus* (Linnaeus, 1758: as *Bufo gibbosus*).



Fig. 8. Lithograph from Daudin (1802: Pl. XX; p.64) of *Hoplobatrachus tigerinus* (Daudin, 1802: as *Rana tigerina*) from "Bengale", in eastern India or Bangladesh.



In the introduction, he wrote of a mix-up in the crediting of the artist, some of the drawings being the work of Jacques (sometimes known as Pierre-Paul) Barraband (1768-1809), a French zoological and botanical illustrator, famous during his time for painting tropical birds. He was hired by Napoleon Bonaparte (1769-1821) to decorate the banquet-hall at Château de Saint-Cloud, his preferred residence. In 1807, Barraband was appointed professor at the school of Arts et Dessin de Lyon (Guinot 2002). In the work, these were credited to another artist, Jacques Eustache de Sève (years active: 1742-1788), who was loaned the artwork, and published the same under his name by mistake. Jacques Eustache de Sève (before 1867-1830) was another French illustrator, famous in his own right and Daudin's wife, Adélaïde (?-1803) contributed images for the work. de Sève was the son of Jacques de Seve (before 1727-1790), who was commissioned by Georges-Louis Leclerc, Comte de Buffon (1707-1788), French polymath (naturalist, mathematician, cosmologist and encyclopaedist) to provide the quadruped illustrations for the 36 volume "Histoire naturelle, générale et particulière" (1749-1778), and subsequently, Buffon's "Recueil de Vingtquatre Plantes et Fleurs" (1772) (Benezit 1911-1923).

Proceedings of the Zoological Society of London (1833-1964)

Issued in octavio format, the Proceedings of the Zoological Society of London commenced publication from 1833, and between 1965 to 1984, was known as the Journal of Zoology: Proceedings of the Zoological Society of London. Throughout the Nineteenth Century, this important English periodical served as the premier venue for publishing papers on taxonomy, especially by workers based at the British Museum and the London Zoo, and by other Anglophone scientists in the colonies. Major illustrators of the journal included the Dutchman, Johannes Gerrardus Keulemans (1842-1912), who concentrated on birds and mammals, while George Henry Ford illustrated most of the text figures of herpetological papers, rendered in both black-and-white lithographic prints, as well as in colour.

A large number of frog species descriptions were published in the Proceedings, and in this section, we illustrate one such work—that of Günther (1875), describing several Indian species, all illustrated by Ford (Figs. 10-11).

Fig. 10. Lithograph from Günther (1876: Plate LXIII) by George Henry Ford, showing (top: left, lateral view; right, ventral view), *Ghataphryne ornata* (Günther, 1876: as *Ansonia ornata*); (middle: left, dorsolateral view; right, dorsal view), *Indirana beddomii* (Günther, 1876: as *Polypedates beddomii*); and (bottom: left, dorsolateral view; right, dorsal view), *Sallywalkerana diplosticta* (Günther, 1876: as *Ixalus diplostictus*).

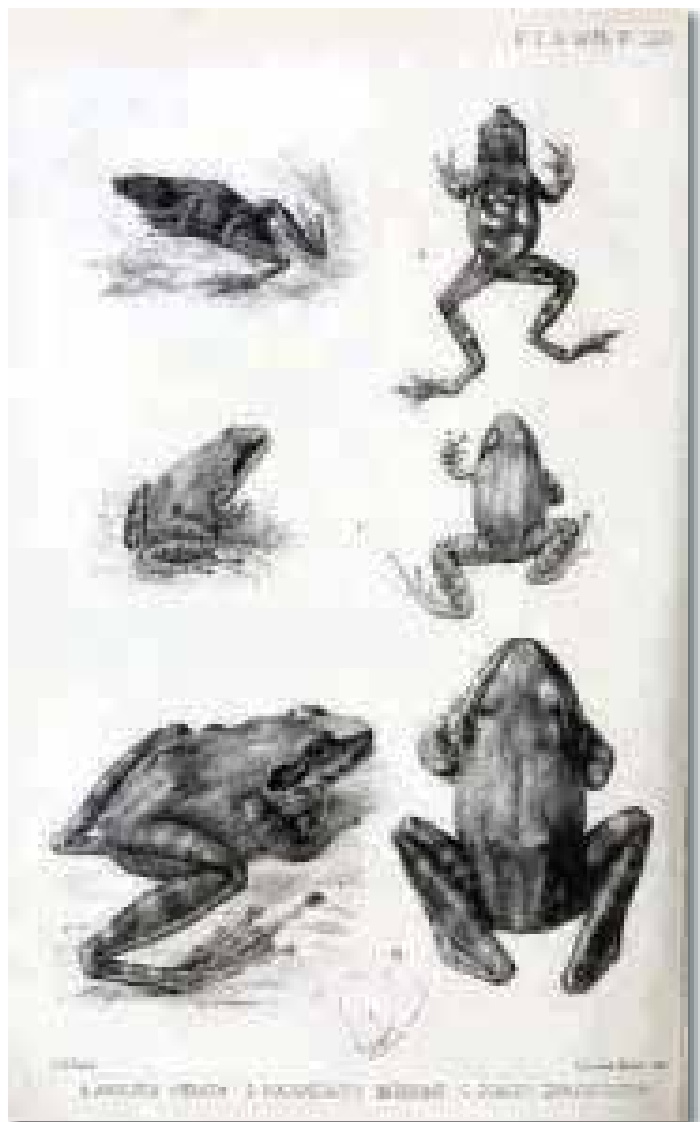
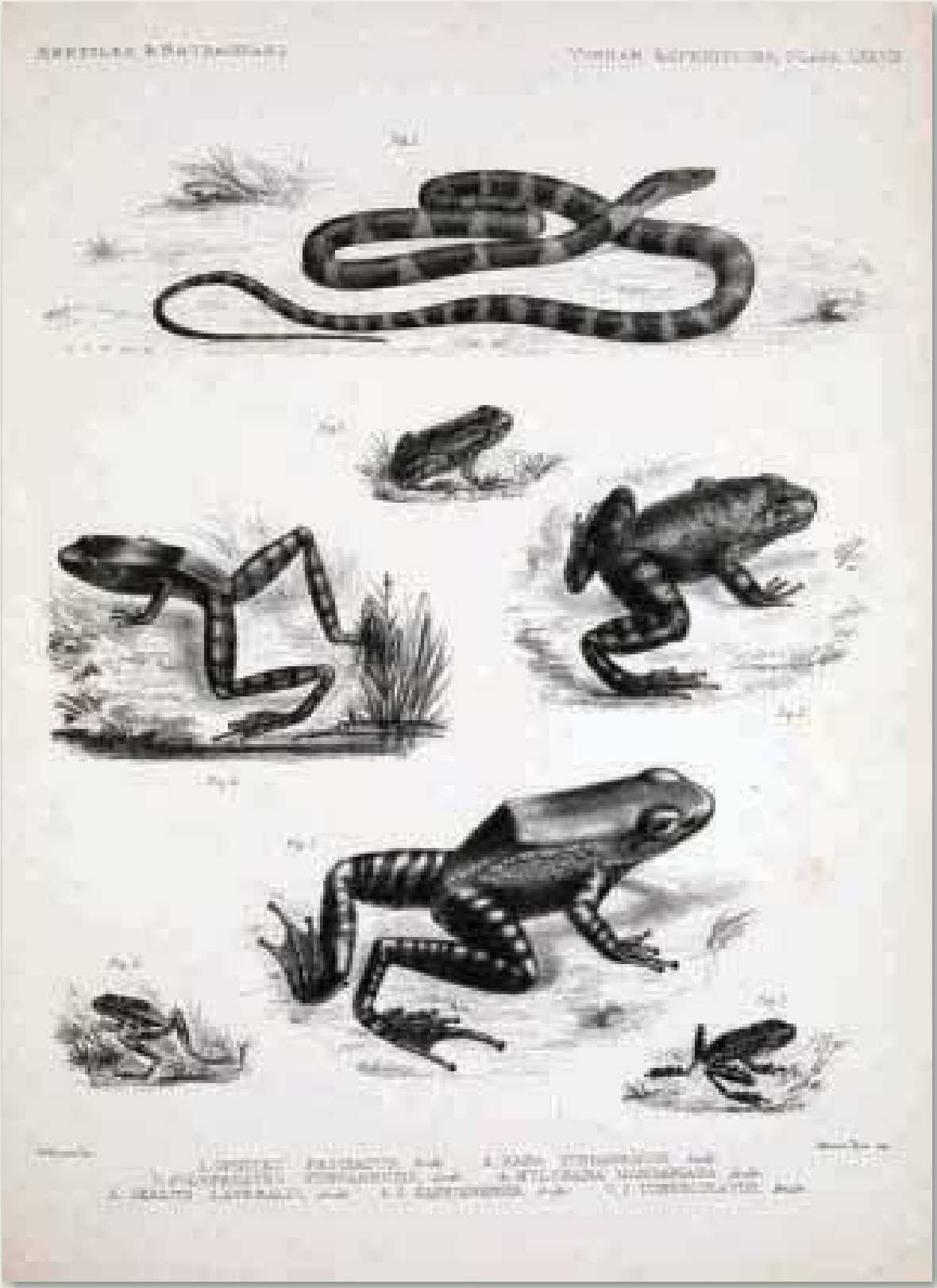




Fig. 11. Hand-coloured lithograph from Günther (1876: Plate LXV) by George Henry Ford, showing (top) *Amolops formosus* (Günther, 1876: as *Polypedates formosus*) and (bottom) *Odorrana chloronota* (Günther, 1876: as *Polypedates chloronotus*).

Anderson's (1879) The Anatomical and Zoological Researches

Fig. 12. Lithographs from Anderson's ("1878" 1879: Plate LXXVIII) Anatomical and zoological researches: *Nanorana yunnanensis* (Anderson, 1879: as *Rana yunnanensis*), *Odorrana andersonii* (Boulenger 1882: as *Polypedates yunnanensis*), "*Hylarana*" *margariana* (Anderson, 1879: as *Hylarana margariana*), *Leptolalax lateralis* (Anderson 1871: as *Ixalus lateralis*); *Amolops afghanus* (Günther 1858: as *Ixalus kakhienensis*) and "*Theloderma*" *andersoni* (Ahl, 1927: as *Ixalus tuberculatus*). On the top of the plate is the snake, *Lycodon fasciatus* (Anderson, 1879: as *Ophites fasciatus*).



After the natural history collection of the Museum of the Asiatic Society of Bengal was donated to the newly established Indian Museum in Calcutta, John Anderson (1833-1900) was hired from England to be the first Superintendent of the Museum. While a medical doctor, with a position at the hospital in Calcutta, Anderson was devoted to zoology and made extensive collections. He was part of both expeditions to Yunnan (1868-1869 and 1874-1875), and produced a work, in quarto, dated 1878 (but published in 1879) that comprised a monograph on the vertebrate fauna of the Upper Burma-Yunnan region (now in the Chinese-Myanmar frontier region). Containing 85 plates, the single plate (Plate LXXVIII: reproduced here as Fig. 12) covering amphibians (shared with a snake) is a black-and-white lithographed one, executed by Robert Mintern (1840-1908). It shows six amphibian species, four of which are now known to occur within Indian limits.

We briefly reviewed a few original descriptions and other early accounts of Indian amphibians, drawing attention of readers to the wealth of early resources available to researchers on the diversity of images presented in these works. Many frog images may appear stiff and somewhat stylized by today's standards. Nonetheless, one should bear in mind that most of the images were executed by European artists, unfamiliar with the fauna and based on long-preserved specimens, dating to the time preceding colour printing and photography.

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Studies on Amphibians of Odisha: An Overview

Abstract

The work presented here is a compilation of information on various studies carried out on the amphibian species in Odisha. First hand information on updated taxonomy, distribution and threats to the species are provided. Distribution of three species of amphibians, namely *Microhyla rubra*, *Philautus sanctisilvaticus* and *Raorchestes terebrans* are reported for the first time from Odisha. The amphibian faunal diversity is represented by 29 species under 2 orders (Anura and Gymnophiona) and 6 families. The anurans are represented by 14 genera comprising 3 species of Bufonids, 11 species of Dicroglossids, 2 species of Ranids, 6 species of Microhylids and 6 species of Rhacophorids. Additionally, one species of Gymnophiona of family Indotyphlidae has recently been described from Eastern Ghats including Odisha. Besides taxonomic and ecological studies sizable literatures are available from studies on biology and applied research related to many amphibian species distributed in the state.

Introduction

Situated in the peninsular India, Odisha is one of the biodiversity rich states because of its varied physiographic and climatic conditions. The physiography of the state is an amalgamation of Chotta Nagpur plateau on the north, Deccan peninsula in west, Eastern Ghats in south and coastal plains towards the eastern side (Rodgers et al. 2003). The dominant forest types in Odisha are of dry deciduous type comprising northern tropical dry deciduous, moist deciduous, mixed forest, coastal mangrove forest and patches of semi-evergreen forests (Meher - Homji, 2001). The varied forest types and ecosystems offer suitable habitat for 29 species of amphibian species distributed in different habitat and ecological landscapes.

The first ever documentations of amphibian fauna of Odisha are from the survey records by Annandale (1915 & 1921). Stray records of amphibians pertaining to Odisha are also available from Boulenger (1890 & 1920). Behura (1965) compiled the information regarding amphibian fauna of Odisha in Odia language, describing four species of

frogs. Subsequent studies by Mohanty-Hejmadi (1976), Mohanty-Hejmadi & Dutta (1976), Dutta (1987a, 1987b, 1997, 1998 & 2003), Dutta & Routroy (1990) and Dutta & Acharjyo (1990) added more number of species to the checklist. Sarkar (1993) recorded 16 species of amphibians based on collection of 2401 specimens from various parts of the state. Various site specific surveys carried out in protected and outside protected areas of Odisha also contributed significantly in knowing the amphibian diversity of the state. Some of the important survey records are by Dutta et al. (2009) from Similipal Biosphere Reserve,

Key words:

Biology, conservation, distribution, ecology, status.

Polypedates cf. teraiensis
and *Chiromantis* sp

Pratyush P. Mohapatra¹, Cuckoo Mahapatra² & Sushil K. Dutta^{3*}

¹Zoological Survey of India, Central Zone Regional Centre, Jabalpur, Madhya Pradesh- 482002, India

²P. G. Department of Zoology, North Orissa University, Takatpur, Baripada, Odisha- 757003, India

³National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), GKVK Campus, Bellary Road, Bengaluru- 560065, India.

Corresponding author e-mail: sushil@biodiversitylab.org



Mohapatra et al. (2010) from Mahendragiri, Deuti & Raha (2010) from Karlapat and Konark-Balukh and wildlife sanctuaries, Sahu et al. (2012) from Hadagarh wildlife sanctuary, Mohapatra et al. (2013) from Nandankanan Wildlife sanctuary, Jena et al. (2013) from Bhitarkanika Mangroves, Sahu et al. (2014) from Gandhamardam Hills, Mishra et al. (2013) from Bolangir district and Rout et al. (2016) from Kuldiha wildlife sanctuary.

Amphibian fauna in the state is also well studied in terms of ecology, breeding biology and ecological energetics which has enlightened our knowledge on various aspects of amphibian biology other than taxonomy. Studies undertaken on these aspects are highlighted under each species. Age determination through skeletochronology technique has been carried out and Dutta et al. (2011) compiled the available information on eight different anuran species distributed in the state. Besides, studies on haematology, physiology and toxicology have also been pursued on many species of amphibians of Odisha. The most remarkable studies on amphibians in Odisha have been in developmental biology, where homeotic transformation of tail to limbs was observed in the tadpoles after the cut end of their tails were exposed to vitamin-A (Mohanty-Hejmadi and Crawford, 2003). Recently, Mahapatra and Mahapatra (2013) gave a detailed account of tail regression in some anuran tadpoles of Odisha. Apart from these works karyotypic work on one species of toad has been carried out in Odisha. Another study by Manna & Bhunya (1966) reported about 22 diploid chromosomes in most of the toads belonging to the genus *Bufo* (= *Duttaphrynus*) in both the sexes, though inconstancy of the chromosome number in various organs of adult and larval toad, *D. stomaticus* has been reported by Sharma, et al. (1965).

Although, great volume of work has been done on the amphibians of Odisha, the biology of many species is still poorly represented. Moreover, the studies are scattered and there was a need for compilation of the reports to give immediate information on various studies undertaken to outline future projects on amphibians of

the state and elsewhere. Hence, the present review gives a lucid account on the studies carried out on amphibians of Odisha and emphasizes the need for extensive studies on their biology.

Amphibian Diversity in Odisha

Amphibian fauna of Odisha is represented by two orders, Anura and Gymnophiona comprising 28 species of anurans and one species of caecilian. Status of each species with existing information on biology of the species are discussed as follows and summarized in Table 1. The nomenclature of amphibians followed here is after Frost (2017).

Order Anura

Till date 28 species of anurans are recorded from Odisha under 5 families and 14 genera comprising 3 species of Bufonids, 11 species of Dicroglossids, 2 species of Ranids, 6 species of Microhylids and 6 species of Rhacophorids.

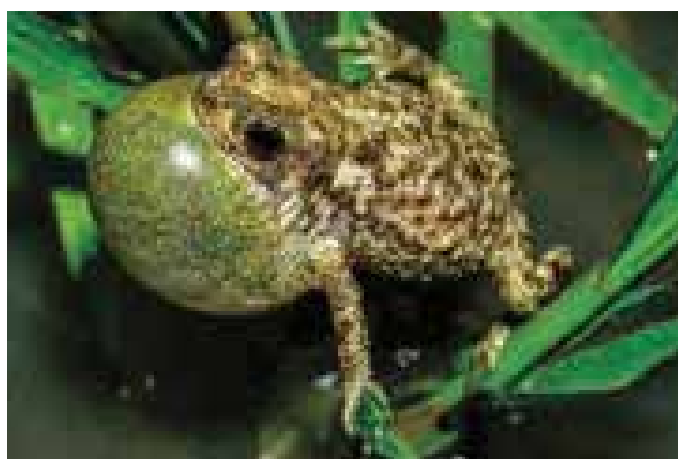
Family Bufonidae

Genus *Duttaphrynus* Frost, Grant, Faivovich, Bain, Haas, Haddad, de Sá, Channing, Wilkinson, Donnellan, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green, and Wheeler, 2006.

Three species of toads under the genus *Duttaphrynus* are distributed in the state. Earlier mentions of *Bufo* for all the toad species of Odisha are now placed under the genus *Duttaphrynus* (see Frost, 2017).

Among the toads, *Duttaphrynus melanostictus* is the most common species

Duttaphrynus scaber



and has a very wide habitat range throughout the state. Consequently, extensive studies on various biological aspects have been carried out on *D. melanostictus*. Studies on feeding habits, breeding biology and development of this species have been carried out by Behura et al. (1972) and Hota & Dash (1983). A very strange case of true hermaphroditism was observed in one specimen of this species from Bhubaneswar by Behera et al. (1971). Behura (1948a) reported *Acacia* thorn in the stomach of a toad and in another report, abnormalities of the oesophageal artery was reported by him (Behura 1948b).

Skeletochronology studies revealed the maximum longevity of this species to be 12 years (Nayak et al. 2007; Dutta et al. 2011). Effects of heavy metals and pesticides on physiology of *D. melanostictus* has also been assessed by various workers (Parida, Kisku et al. 2014; Parida, Mangaraj et al. 2014; Parida, Naik et al. 2014; Parida, Pal et al. 2014; Parida, Tudu et al. 2014; Parida, Naaz et al. 2015; Parida and Pattanayak, 2015; Pattanayak and Parida, 2014). Similarly, studies on tadpoles of *D. melanostictus* revealed that lysosomal enzymes and melanocytes act as key players in bringing about cell death during tail regression, an important event during metamorphosis (Mahapatra and Mahapatra, 2012; 2013; 2015a & b). Another study by Mahapatra et al. (2011) on two populations of *D. melanostictus* proposed that, apart from environmental factors, variations in the activity of lysosomal enzyme-acid phosphatase also mediates variations in the duration of larval period. Extraneously applied thyroxine showed toxic and teratogenic effects on the tadpoles of *D. melanostictus* and the effects were dose and stage specific (Mahapatra et al. 2015). Das and Dutta (1996) reported the development of ectopic supernumerary limbs in the tadpoles of *D. melanostictus* from cut end of tails exposed to vitamin-A.

Duttaphrynus scaber was first recorded from Odisha by Dutta (1987a) based on specimens collected from Sambalpur by Dr D. Platt during 1955-57. Later, this species was reported from Balugaon and other coastal districts of the state. Earlier mention of *Bufo andersonii* (a junior synonym of *D. stomaticus*) from Sambalpur by Mohanty-



Hylarana tytleri

Hejmadi (1976) actually corresponds to *Duttaphrynus scaber* (vide Dutta, 1987a). Dutta et al. (2009) mentioned distribution of the species throughout the state and this species is mostly restricted to agricultural fields and scrub forests.

Duttaphrynus stomaticus was first reported from the state by Dutta (1987a) from Sambalpur and Brajarajnagar. Later, this species was recorded from northern Odisha in Similipal, Thakurmunda (Dutta et al. 2009). The authors recorded small populations near Dhenkanal railway station and in Kamakhya Nagar. This species has patchy distribution in Odisha, living in sympatry with *D. melanostictus*. Parida et al. (1988) reported 22 diploid chromosomes in this species. Studies on growth and development of tadpoles of this species have been carried out by Dash and Mahapatro (1990), Hota et al. (1991), Mahapatro and Dash (1987, 1990, 1991a, 1991b, 1992).

Family Dicroglossidae

This family is represented by 11 species under 4 genera such as *Euphlyctis* (2 species), *Fejervarya* (4 species), *Hoplobatrachus* (2 species) and *Sphaerotheca* (3 species).

Genus *Euphlyctis* Fitzinger, 1843

This genus is represented by two species, namely *E. cyanophlyctis* and *E. hexadactylus*. The Indian skittering frog (*E. cyanophlyctis*) is one of the most common frogs found in all kinds of water bodies. Many colour morphs, such as olive green with dark blotches, reddish without any

marking, brownish and dark grey are observed in various populations of this species. Mohanty-Hejmadi and Dutta (1979) studied the breeding biology of this species. The maximum age of *E. cyanophlyctis* was recorded to be 6 years and sexual maturity was attained at the age of two years (Dutta et al. 2011). The other most conspicuous frog of this genus is the green pond frog (*E. hexadactylus*). This species was first recorded from the state by Dutta and Routray (1990) and the authors gave detailed description about ecology and natural history of the species. This species is distributed along the coastal districts of Odisha starting from Ganjam in the south to Balasore in the north and has also been recorded from Cuttack (Choudwar, Anshupa lake), Khurdha, Bhadrak and Jajpur districts. Jena et al. (2013) reported this species from Bhitarkanika mangrove ecosystems. Singh and Dutta (1995) has discussed about various problems and suggested remedies for conservation of this species. Maximum age of this species has been reported to be at least 14 years in nature, whereas maturity in both males and females were attained at the age of two years (Nayak et al. 2008 & Dutta et al. 2011).

Genus *Fejervarya* Bolkay, 1915

The genus *Fejervarya* is represented by three species and one undescribed species, with certainty (pers. obs.). Of these, *Fejervarya syhadrensis* is most common and widespread, occurring in varied habitat types. The earlier records of *Fejervarya limnocharis* (= *Limnonectes limnocharis*) corresponds to *F. syhadrensis*, as the distribution of the former species is restricted to South East Asia (Frost, 2017). Several studies have also been carried out on the species under the nominal taxon *L. limnocharis*. Dutta and Singh (1997) discussed about status of *Limnonectes limnocharis* species complex in Asia and Mohanty et al. (1995) reported polymorphism and inheritance of mid-dorsal strip in the species hitherto referred as *Rana limnocharis*. Similarly study on breeding and life history of *L. limnocharis* was carried out by Mohanty et al. (1996) and later Mohanty et al. (1997) reported the population dynamics and growth of the species in natural conditions in Odisha. There are also reports of homeotic

transformation of tails to limbs on exposure to vitamin A in this species (Das & Dutta, 1996).

Another species under this genus is Dutta's cricket frog (*Fejervarya orissaensis*), which is widely distributed throughout the state but mostly confined to the agricultural fields. Dutta (1997) described the species as *Limnonectes orissaensis* based on specimens collected from Bhubaneswar, Balasore and Sambalpur.

The most interesting species of this group is the crab-eating frog (*Fejervarya moodiei*), which was earlier known by the name *F. cancrivora*. This species is distributed in coastal Odisha in the brackish water habitats of Balasore coast, Bhitarkanika, Dhamra and Chilika Lagoon.

It is presumed that the genus *Fejervarya* is represented by more numbers of taxonomically cryptic taxa in Odisha and in this paper we report occurrence of a most distinct form of undescribed species. Besides taxonomic studies, skeletochronology in *Fejervarya* sp. in Odisha revealed the maximum age to be four years and age of sexual maturity to be two years (Dutta et al. 2011).

Genus *Hoplobatrachus* Peters, 1863

The genus *Hoplobatrachus* comprises two species, i.e., the Indian Bull frog and the Jerdon's bull frog and both the species are distributed in Odisha. Apart from these two species there is an isolate population of *Hoplobatrachus* in Mayurbhanj district having external resemblance with *H. crassus* but differs from the later in colouration, call pattern and genetics.

The Indian bull frog breeds in temporary pools and the males become yellowish in colour during breeding season. The mating takes place during night time and continues till morning in rainy days. Dutta & Mohanty-Hejmadi (1976; 1981) studied the breeding, life history, sex ratio and size correlation of *H. tigerinus* and studies on larval biology were carried out by Dash & Hota (1980), Hota (1981) and Mohanty & Dash (1986). Some studies on effect of pesticides on the tadpoles of this species were also under taken by Dutta (1995), Dutta & Mohanty-Hejmadi (1978) and Mohanty-Hejmadi & Dutta (1981). Similarly, studies on larval

energetics of the species have been carried out by Hota (1986), Mohanty and Dash (1988). Inter and intra specific predation of *H. tigrinus* tadpoles was recorded by Mohanty-Hejmadi & Dutta (1981) and Hota & Dash (1983); the later study reporting predation of Indian bull frog tadpoles on *D. melanostictus* larvae. Mitra (1975) reported predation on adult *H. tigrinus* by giant water bug (*Belostoma indicum*). Homeotic transformation of cut end of tails to limbs upon exposure to vitamin A has also been observed in the tadpoles of *H. tigrinus* (Das & Dutta, 1996). Recently, there have been reports on tail regression (Mahapatra et al. 2012) and toxicological effects of pesticides on the tadpoles of *H. tigrinus* (Parida, Biraja et al. 2015; Parida, Jena et al. 2015). With reference to the Jerdon's bull frog Dutta et al. (1992) studied sexual dimorphism in the species and later in 1994 the authors studied the breeding and development of this species.

Genus *Sphaerotheca* Günther, 1859

The genus *Sphaerotheca* was earlier placed under the genus *Rana* and *Tormopterna* and is represented by three species in Odisha such as *Sphaerotheca breviceps*, *S. dobsonii* and *S. rolandae*. All these species are burrowing in habit and are mostly seen during breeding season, commencing from June to October, i.e. monsoon to post monsoon. The short-headed burrowing frog (*S. breviceps*) and the Indian burrowing frog (*S. rolandae*) are distributed throughout the state. Dutta (1988b) reported Dobson's burrowing frog (*S. dobsonii*) from Odisha and this species has been recorded from Mayurbhanj, Keonjhar, Cuttack, Khordha, Angul, Nayagarh and Kalahandi districts (Dutta et al. 2009 & pers. obs.). Das et al. (1996) studied population dynamics and growth while Dutta et al. (2005) analyzed the clutch and body size of the Indian burrowing frog. Das and Dutta (1996) also reported the formation of odd number of ectopic limbs in the tadpoles of *Tormopterna rolandae* (= *S. rolandae*) in their homeotic transformation experiments. Similarly, Mohanty-Hejmadi et al. (1979) studied the life history of the short-headed burrowing frog.

Family Ranidae

In Odisha, this family is represented by two genera (*Hydrophylax* and *Hylarana*) comprising two species.

Genus *Hydrophylax* Fitzinger, 1843

The earlier report of *Hylarana malabarica* from Sambalpur, Similipal, Keonjhar, Dhenkanal, Angul, Nayagarh and Khurdha districts are now placed under *Hydrophylax bahuvistara* (See Padhey et al. 2015). Sarkar (1993) recorded the species from Odisha, based on two specimens collected from Badrama and Bhawanipatna and provided a comparison between the two morphs. Kar et al. (2011) recorded predation of an adult fungoid frog by a giant water bug.

Genus *Hylarana* Tschudi, 1838

One species of frog under this genus has been known to occur in Odisha, namely *Hylarana tytleri*, which was earlier reported as *Rana erythraea* from Bhubaneswar and Cuttack (Mohanty-Hejmadi, 1997; Sarkar, 1993). This species has been further recorded from Kendrapada, Jagatsinghapur, Puri, Khurdha and Cuttack districts of Odisha. Frost (1997) mentioned about *H. taipehensis* from Odisha, which probably corresponds to *H. tytleri*.

Family Microhylidae

This family is represented by six species in the state under two genera *Uperodon* (4 species) and *Microhyla* (2 species).

Genus *Uperodon* Duméril and Bibron, 1841

Earlier names of *Kaloula taprobanica* and *Ramanella variegata* are now synonymised under genus *Uperodon*. Dutta (1987b) recorded *Kaloula pulchra* from Konark-Balukhand area of Odisha which actually corresponds to *Uperodon taprobanicus* (= *Kaloula taprobanica*).

Life history study on marbled balloon frog (*Uperodon systoma*) was carried out by Mohanty-Hejmadi et al. (1979a). A famous experiment conducted in the Cell and Developmental biology laboratory of Utkal University, Odisha was the first report of homeotic transformation in vertebrates where extra limb generation was observed in marbled balloon frog, *U. systoma* at site of tail amputation after vitamin-A treatment (Mohanty-Hejmadi et al. 1992). Later,

homeotic transformation was also reported in the tadpoles of *M. ornata* and other anurans as discussed above (Das and Dutta, 1996).

Genus *Microhyla* Tschudi, 1838

Two species, namely ornate narrow-mouthed frog (*Microhyla ornata*) and red narrow-mouthed frog (*M. rubra*) are distributed in the state, of which the former is common throughout. The red narrow-mouthed frog is reported for the first time from the state based on photographic evidences and collection of voucher specimens. The species has been recorded from South Odisha, specifically in Nayagarh (Baisipalli WLS), Ganjam and Phulbani districts. Khan et al. (1979), Mohanty-Hejmadi et al. (1980); Dei et al. (1994) and Dash and Dei (1998) have given detailed account on life history of the ornate narrow-mouthed. Dey et al. (1989) discussed about chondrification and osteological development of vertebral column in the tadpoles of the species through differential staining and mentioned that the chondrification as well as ossification occurs proximo-distally. The maximum age of *Microhyla ornata* was found to be four years by skeletochronology studies and although the females attained sexual maturity at two years, males became sexually mature at one year (Dutta et al. 2011). Recently, Hota et al. (2013) published the blood cell profile of developing tadpoles and adults of ornate frog, *Microhyla ornata*.

Family Rhacophoridae

This family is represented by six species under three genera, such as *Chiromantis* (1 species), *Philautus* (2 species), *Raorchestes* (1 species) and *Polypedates* (2 species).

Genus *Chiromantis* Peters, 1854

The genus *Chiromantis* (= *Chirixalus*) was first reported by Dutta et al. (2009) from Similipal, Balasore, Dhenkanal, Cuttack, Khordha, Nayagarh and Ganjam districts of Odisha, which corresponds to *Chiromantis simus*. Age estimation studies in *Chiromantis simus* show the maximum age of the species to be four years in nature (Dutta et al. 2011). Das and Mahapatra (2016) have recently described the blood cell profile of developing tadpoles of *Chiromantis simus*.

Genus *Philautus* Gistel, 1848 and

Raorchestes Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010

Dutta (2003) discovered *Philautus similipalensis* from Similipal Biosphere Reserve based on specimens studied from the Reserve and later this species was recorded from Mayurbhanj, Khurdha, Dhenkanal, Ganjam, Gajapati and Rayagada districts. The other two species of bush frogs recorded for the first time from Odisha are *Raorchestes terebrans* from Koraput and Mahendragiri and *Philautus sanctisilvaticus* from Potangi, Koraput district based on specimens collected from these localities. *R. terebrans* and *P. sanctisilvaticus* are reported for the first time from the state by the authors. Deuti et al. (2014) also reported *P. sanctisilvaticus* from Araku valley, Andhra Pradesh, which is close to Potangi hills.

Genus *Polypedates* Tschudi, 1838

This genus is represented by two species, namely Indian tree frog (*Polypedates maculatus*) and six-lined tree frog (*P. teraiensis*). Among these species the former is widely distributed in the state where as the later has a patchy distribution in Mayurbhanj, Cuttack and Khurdha districts of Odisha (Dutta et al. 2009). Dutta et al. (2001) studied breeding and development of *P. maculatus* and reported the clutch size of 107-678 with hatching success of 90%. Das and Mahapatra (2012 and 2015) have elaborated the blood cell profile of developing tadpoles of *Polypedates teraiensis* and *P. maculatus*. The haematology of adult *P. maculatus* (Mahapatra et al. 2012) and *P. teraiensis* has also been worked out by Das and Mahapatra (2014). Age estimation studies in *Polypedates teraiensis* shows the maximum age of the species to be five years (Dutta et al. 2011). Numerous studies on vitamin A mediated tail regeneration have been done on rhacophorids especially *P. maculatus* (Mahapatra et al. 2001; Mohanty-Hejmadi and Crawford, 2003; Mahapatra et al. 2004). Recently, Mahapatra et al. 2017 studied the immunohistochemical localization of acid phosphatase in non-amputated, normally regenerated and vitamin A treated abnormally regenerated tails (a prerequisite for ectopic organ development) of tadpoles of *P. maculatus*. Studies on tail regression in tadpoles of *P. maculatus* revealed the involvement of cathepsin D as an important

enzyme for causing cell death in the resorbing tail (Mahapatra and Mahapatra, 2011). Inter-specific variations in the duration of tail regression in *D. melanostictus*, *P. maculatus* and *H. tigerinus* were also attributed to the differential levels of cathepsins in the regressing tails (Mahapatra et al. 2012; Mahapatra and Mahapatra, 2013).

Order Gymnophiona

Family Indotyphlidae

The Order gymnophiona is represented by one family and a recently described species from Eastern Ghats, named *Gegeneophis orientalis*. This species is the first caecilian reported from the state of Odisha, the first teresomatan caecilian from the Eastern Ghats, and is the only Indian indotyphlid known from outside the Western Ghats region. The Eastern *Gegeneophis* is known only from above 1,100 m in habitats with canopy cover and most preferably in riparian zones. The species appears to be tolerant of some anthropogenic disturbance, because it was locally abundant in coffee estates, but it has not been found where the canopy is open, and much forest habitat has been lost (Agarwal et al. 2013). Till now the only known habitat of this caecilian species is at Deomali in Odisha, which is being degraded rapidly through large-scale livestock grazing and fuel wood collection, and the hills in Koraput District are facing threats from mining and monoculture social forestry activities. Nothing is known about the biology and ecology of this species.

Conservation of Amphibians in Odisha

Major predicted threats to the amphibian fauna are habitat loss, pollution, poaching, diseases and climate change (Stuart et al. 2004). Impact of pollution due to pesticides is a well known negative factor affecting all aquatic life including amphibians, which has been highlighted by various workers (Mann et al. 2009; Khangarot and Ray, 1987; Birge et al. 2000). Broadly, pollution is the impact of anthropogenic activities and water pollution directly affects the amphibian population causing various genetic and morphogenetic deformities. Sometimes the overdose cause complete wiping out of the population as observed in several agro-ecosystems (pers. obs.).

Industrial effluents drained to aquatic ecosystem, eutrophication, and washed off agricultural pesticides are some of the major threats to the aquatic amphibian species (Broomhall, 2004). Thus, amphibians are also good bio-indicators of pollution. Dutta (1997) also discussed about various threats and conservation measures for amphibians of north-eastern Orissa, with special reference to Similipal.

Similarly, habitat degradation or loss due to conversion of wetlands to human habitation and agricultural fields are equally major threats for survival of many amphibian species. Although reports on amphibian poaching cases in Odisha are negligible, many of the amphibians (mostly the *Hoplobatrachus* species and the green pond frog) are consumed for their meat. However, from unofficial sources poaching of frogs still continues by some specific communities in rural and urban Odisha though no commercial exploitation has been noticed. In traditional medicine, meat of *Hoplobatrachus tigerinus* is believed to have zootherapeutic use to cure asthma. It is also pertinent here to mention that some of the frogs of genus *Rana* (now placed under genera *Euphlyctis* and *Hoplobatrachus* of Family Dicroglossidae) are listed in Schedule - IV of Wildlife (Protection) Act, 1972. In addition, the export of these species requires permits under CITES and the Indian Wildlife (Protection) Act, 1972.

There are some records of diseases due to fungal infection and nematode parasites among amphibians in India (Dhanukar et al. 2013; Molur et al. 2015). However, impacts of such diseases are not studied in Odisha. Furthermore, diseases are often climate related, so surveillance and monitoring of such diseases in nature will be effective to control any epidemic in future.

Studies conducted elsewhere in the world has shown that many frog populations are forced towards extinction, seemingly from climate warming (Pounds & Crump, 1994; Pounds et al. 1999) and for tropical species of amphibians rainfall seems to be the most significant climatic factor (Bickford et al. 2010). Climate change along with other habitat related threats have visible impact on some of the amphibian species in Odisha which are yet to be reported. Studies have also shown that many amphibian species

have adapted to prolonged breeding period and skip hibernation.

Many of the ecological assessment projects made for clearance of developmental activities have underestimated the actual species diversity either in favour of the companies or due to ignorance. Similarly road traffic poses some serious threats on amphibian species as the road kill incidents increases and many frog and toad populations might completely cut-off from the nearby populations (Fahrig et al. 1995; Vijaykumar et al. 2001). Hence developmental projects without scientific validation of impact assessments severely affect the local fauna. Furthermore, with the advancement of modern techniques and scientific thought a better management strategy can be developed without affecting much to the ecosystem.

Apart from the continuous taxonomic resurrections in amphibian species, there are several erroneous records and incomplete reports with reference to amphibian fauna of the state. Murthy (1987) reported six species of amphibians from Chilka lagoon, which was with many typological errors (repeat of the species name *Rana breviceps*; typological error in the name Bufo and record of *Rhacophorous leucomystax*). Sarkar et al. (1993) reported *Rana keralensis* for the first time from Odisha from Simlipal and mentioned that the species is rare in the state, which probably corresponds to *Fejervarya orissaensis*. Similarly, survey report of Rout et al. (2016) from Kuldiha wildlife sanctuary is with taxonomic errors and probably due to incomplete survey the mentioned amphibian species diversity suffers from underestimated species diversity.

Conclusion

Current knowledge on amphibian species diversity and distribution in Odisha still need more systematic approach. With increasing popularity of molecular taxonomy, the rate of describing cryptic species has increased many folds. Hence, it is believed that modern taxonomic tools will be of utmost use in resolving the taxonomy of the cryptic amphibian species of the state. The current compilation also indicates enormous scope to undertake studies on ecology, toxicology, physiology; developmental biology and ethology since the basic biology of the amphibians found in Odisha is still unknown. Sincere conservation efforts and long term surveys to assess and mitigate various levels of threats including climate mediated changes on amphibians are some of the challenges to be addressed.

Acknowledgements

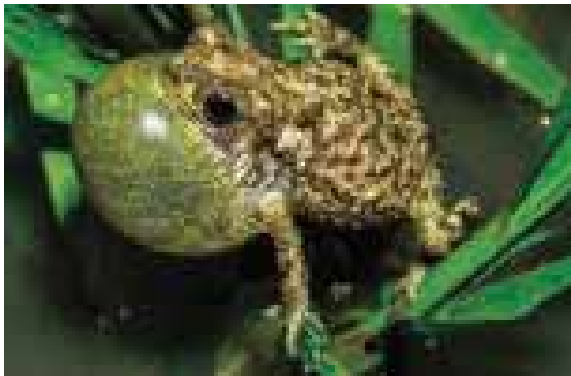
The authors express their deep sense of gratitude to the PCCF and Chief Wildlife Warden, Odisha along with other staff members of state forest department for logistic support to carry out surveys in various forested areas of the state. PPM is extremely thankful to Dr K. Chandra, Director, Zoological Survey of India and Dr S. Sambath, Officer-in-Charge, ZSI, CZRC, Jabalpur for encouragement and support to complete the manuscript. We also express our deep sense of gratitude to the forest officers of Odisha, especially Dr Manoj V Nair; Dr Arun K. Mishra, Dr Akshya Pattnaik and Mr. Manoj K. Mohapatra for extending generous help during field studies. We are extremely thankful to our friends such as Dr Abhijit Das (WII, Dehradun), Vivek Sarkar, Himanshu S. Palei, S. K. Sajan, Dr Prasad K. Dash, Dr Satyanarayan Mishra and Asish K. Das for their help during field surveys.

PLATE-1

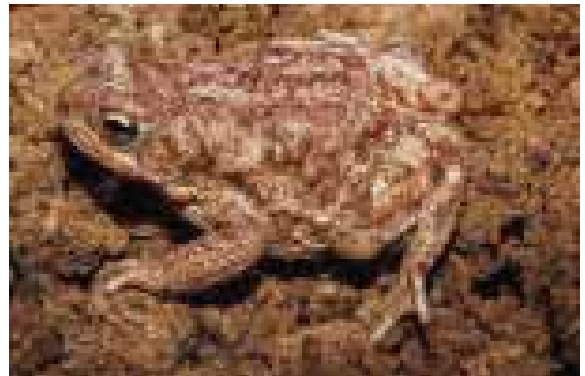
Duttaphrynus melanostictus, Bhuvaneshwar



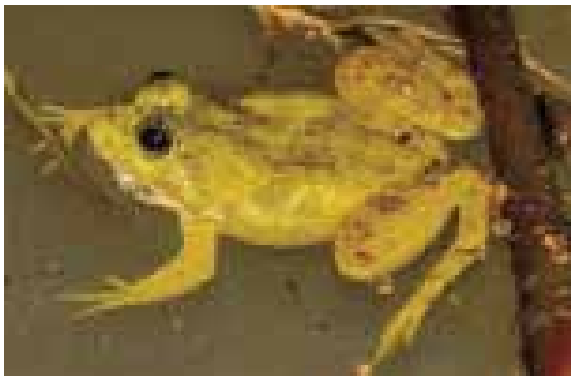
Amplecting pair of *Duttaphrynus stomaticus*, Sambalpur



Duttaphrynus scaber male, Bhuvaneshwar



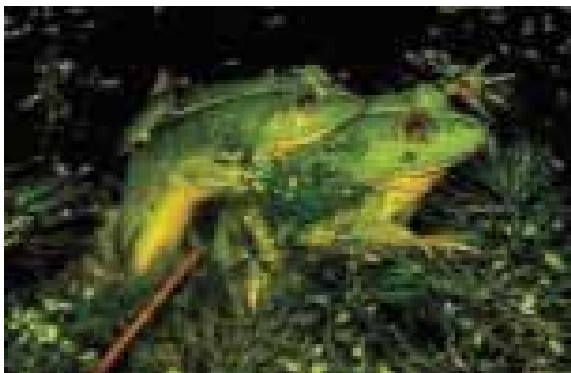
Duttaphrynus scaber female, Dhenkanal



Euphlyctis cyanophlctis, Ganjam



Euphlyctis cyanophlctis, Baisipalli WLS



Amplecting pair of *Euphlyctis hexadactylus* from Ganjam



Euphlyctis hexadactylus, Bhitarkanika WLS

PLATE-2



Fejervarya orissaensis, Baisipalli WLS



Fejervarya orissaensis male, Choudwar, Cuttack



Fejervarya syhadrensis, Sambalpur



Fejervarya sp., Barbara



Fejervarya moodiei, Bhitarkanika WLS



Hoplobatrachus tigerinus, Koraput



Hoplobatrachus crassus, Ganjam



Amplecting pair of *Hoplobatrachus crassus*, Mayurbhanj

PLATE-3



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PLATE-4



Uperodon globulosus, Mayurbhanj



Uperodon systoma, Bhubaneswar



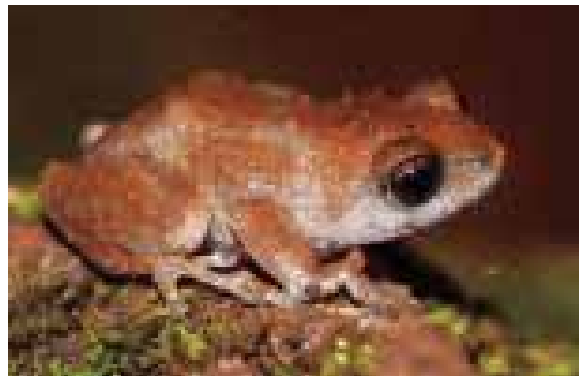
Polypedates maculatus, Angul



Polypedates teraiensis, Barbara, Khurdha



Chiromantis simus, Kalinga, Phulbani



Philautus sanctisilvaticus, Gupteswar, Koraput



Raorchestes terebrans, Potangi hill, Koraput



Gegeneophis orientalis, Deomali, Koraput

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Amplexing pair of *Duttaphrynus stomaticus*

Table 1. Diversity, distribution and conservation status of amphibians of Odisha

S. No.	Taxon	Taxonomic remarks	Distribution	Status		Threats & Conservation
				IUCN	WLPA	
Order Anura						
Family Bufonidae						
1	<i>Duttaphrynus scaber</i> (Schneider, 1799)	Earlier records of <i>Bufo fergusonii</i> , <i>B. scaber</i> and <i>B. andersonii</i> from Odisha refer to this species.	Throughout the state	LC	NL	As this species is most common in agricultural fields, use of excessive pesticide may have serious impact on the population
2	<i>Duttaphrynus stomaticus</i> (Lütken, 1864)	Earlier report of <i>Bufo stomaticus</i> refers to this species.	Mayurbhanj, Keonjhar, Sambalpur, Dhenkanal districts	LC	NL	Patchy distribution and may be occurring in low population.
3	<i>Duttaphrynus melanostictus</i> (Schneider 1799)	Earlier records of <i>Bufo melanostictus</i> refers to this species.	Throughout the state	LC	NL	No major threats
Family: Dicroglossidae Anderson, 1871						
4	<i>Euphlyctis cyanophlyctis</i> (Schneider, 1799)	Earlier reports of <i>Rana cyanophlyctis</i> and <i>R. keralensis</i> from Odisha refer to this species.	Throughout the state	LC	IV	There is no major threat to the species
5	<i>Euphlyctis hexadactylus</i> (Lesson, 1834)	Earlier records of <i>Rana hexadactyla</i> refers to this species.	Coastal districts	LC	IV	Meat consumption, habitat loss/ degradation, excessive use of pesticide.

S. No.	Taxon	Taxonomic remarks	Distribution	Status		Threats & Conservation
				IUCN	WLPA	
6	<i>Fejervarya orissaensis</i> (Dutta, 1997)	Earlier record of <i>Limnonectes orissaensis</i> belongs to this species	Throughout the state	LC	NL	Use of pesticide is the major threat to the species.
7	<i>Fejervarya syhadrensis</i> (Annandale, 1919)	Earlier reports of <i>Limnonectes limnocharis</i> belongs to this species.	Throughout the state	LC	NL	Use of pesticide is the major threat to the species.
8	<i>Fejervarya moodiei</i> (Taylor, 1920)	Earlier records of <i>Rana cancrivora</i> and <i>Fejervarya cancrivora</i> are considered to be this species.	Along the east coast in Bhitarkanika, Kendrapada, Balasore, Bhadrak and Chilika	DD	NL	Habitat destruction and degradation are some of the common threats in its distribution range in Odisha.
9	<i>Fejervarya</i> sp.	Taxonomic status of this frog population is pending but from preliminary molecular and morphological investigation, it is inferred that this species is new to science.	Similipal, Satkosia, Nayagarh, Phulbani, Gajapati, Koraput and Kalahandi			No immediate threat envisaged. In Koraput, this species was found in agricultural fields and use of pesticides might have adverse impact on the population.
10	<i>Hoplobatrachus crassus</i> (Jerdon, 1854)	An interesting colour morph has been recorded from an isolated patch in Northern Odisha, which warrants detailed taxonomic investigation.	Throughout the state.	LC	IV	Habitat degradation and pesticide.
11	<i>Hoplobatrachus tigerinus</i> (Daudin, 1802)	Earlier records of <i>Rana tigrina</i> and <i>R. tigerina</i> refer to this species.	Throughout the state	LC	IV	Major threats are from poaching for consumption of meat (also ethnozoological value), habitat degradation and pesticide.
12	<i>Sphaerotheca breviceps</i> (Schneider, 1799)	Earlier records of <i>Rana breviceps</i> and <i>Tomopterna breviceps</i> refer to this species.	Throughout the state.	LC	NL	No major threats envisaged.
13	<i>Sphaerotheca dobsonii</i> (Boulenger, 1882)	Earlier records of <i>Tomopterna dobsonii</i>	Mostly in Sal forests of the state.	LC	NL	No major threats envisaged.
14	<i>Sphaerotheca rolandae</i> (Dubois, 1983)	Earlier records of <i>Tomopterna rolandae</i> refer to this species.	Throughout the state.	LC	NL	No major threats envisaged.
Family: Ranidae Rafinesque-Schmaltz, 1814						
15	<i>Hydrophylax bahuvistara</i> Padhye, Jadhav, Modak, Nameer, and Dahanukar, 2015	Earlier report of <i>Rana malabarica</i> and <i>Hydrophylax malabarica</i> from Odisha refer to this newly described species.	Throughout the deciduous forests of the state.	NE	NL	No immediate threats envisaged. This species naturally occur in low population.

16	<i>Hylarana tytleri</i> Theobald, 1868	Earlier records of <i>Rana tpehensis</i> by Dutta & Acharjyo (1990) and <i>Rana erythraea</i> by Sarkar (1993) from Odisha corresponds to this species. Two distinct colour morphs, green and brown are observed in the state, warranting taxonomic investigation.	Kendrapada, Jagatsinghapur, Puri, Khurdha and Cuttack districts.	LC	NL	Habitat loss due to clearing of floating aquatic vegetations and clearing of weeds around the water bodies are some common threats to the species. Run-off residual pesticides might have deleterious impact on this species.
Family: Microhylidae Günther, 1858						
17	<i>Microhyla ornata</i> (Dumeril and Bibron, 1841)	Two distinct morphotypes are observed in the state, warranting taxonomic investigation.	Throughout the state.	LC	NL	Conversion of wetlands in to human habitation and use of pesticides are some of the threats to the species.
18	<i>Microhyla rubra</i> (Jerdon, 1854)	Sexually dichromatic species, needs validation.	South Odisha, specifically in Nayagarh (Baisipalli WLS), Ganjam and Phulbani districts.	LC	NL	This species naturally occur in low population. In the known distribution localities small scale sand mining might have adverse impact on the population.
19	<i>Uperodon taprobanicus</i> (Parker, 1934)	Earlier records of <i>Kaloula taprobanica</i> and <i>K. pulchra</i> from Odisha refer to this species.	Throughout the state including mangrove forests.	LC	NL	Habitat loss due to cutting of trees with holes and buttresses are threats to this species. Use of pesticides might have adverse impact on the larvae as well as adults.
20	<i>Uperodon variegatus</i> (Stoliczka, 1872)	Earlier records of <i>Ramanella variegata</i> refers to this species.	Throughout the state.	LC	NL	Use of pesticides in agricultural fields might be adversely impacting the population.
21	<i>Uperodon globulosus</i> (Gunther, 1864)	Possibility of hybrid population between <i>U. systoma</i> and <i>U. globulosus</i> observed in Odisha from Mayurbhanj.	Throughout the state.	LC	NL	In some areas the early breeders suffer from quick drying of temporary water bodies and hence cause mass mortality of tadpoles.
22	<i>Uperodon systoma</i> (Schneider, 1799)	Same as above.	Throughout the state.	LC	NL	Same as above.
Family: Rhacophoridae Hoffman, 1932						
23	<i>Chiromantis simus</i> (Annandale, 1915)	Earlier record of <i>Chirixalus</i> sp. and <i>Chiromantis</i> sp. from Odisha	Mayurbhanj, Dhenkanal, Angul, Cuttack,	LC	NNL	Habitat loss and infection due to a flesh fly are some of the known threats to this

S. No.	Taxon	Taxonomic remarks	Distribution	Status		Threats & Conservation
				IUCN	WLPA	
		are now placed under the said species.	Khurdha, Ganjam, Gajapati			species.
24	<i>Philautus similipalensis</i> Dutta, 2003	Several morphs are recorded from the state, warranting in-depth taxonomic study.	Similipal (Mayurbhanj dist.), Kuldiha (Balasore dist.), Kapilas (Dhenkanal dist.), Barbara (Khurdha dist.)	LC	NL	No major threats envisaged.
25	<i>Philautus sanctisilvaticus</i> Das and Chanda, 1997	New record from the state.	Koraput.	CR	NL	No major threats envisaged.
26	<i>Raorchestes terebrans</i> (Das and Chanda, 1998)	New record from the state. Earlier known from the type locality Vishakhapatnam, Eastern Ghats, Andhra Pradesh.	Koraput and Gajapati districts.	DD	NL	No major threats envisaged.
27	<i>Polypedates maculatus</i> (Gray, 1830)	Earlier records of <i>Rhacophorous maculatus</i> refers to this species.	Throughout the state.		NL	Nest predation due to a flesh fly for the individuals breeding during post-monsoon seasons (August-September) is the major threat to this species.
28	<i>Polypedates teraiensis</i> (Dubois, 1987)	Confusion related to the exact distribution status of this species in Peninsular India.	Mayurbhanj, Cuttack and Khurdha districts.		NL	Habitat loss/ degradation might have adverse impact on the species.

Order: Gymnophiona

Family: Indotyphlidae Lescure, Renous, and Gasc, 1986

29	<i>Gegeneophis orientalis</i>	Newly described species from Eastern Ghats and Odisha.	Deomali	NE	NL	Habitat loss, deforestation, fishing activities near the stream, forest fire, collection of tubers and other livelihood dependency from the forest. This species has only been recorded from non-protected area in the state, so Deomali should be declared as a biodiversity heritage site considering the unique biodiversity.
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NE: Not evaluated by IUCN; NL: Not listed in Indian Wild Life (Protection) Act, 1972



Validity of the nomen *Polypedates himalayensis* (Annadale, 1912)

Abstract

The present study confirms the species status of *Polypedates himalayensis* and suggests its removal from the synonymy of *Polypedates leucomystax* and *Polypedates maculatus*. Further the study proposes the occurrence of two distinct species group within *Polypedates*, namely *leucomystax* (represented by *P. teraiensis*), and an intermediate between *leucomystax* and *maculatus* (represented by *P. himalayensis* and *P. megacephalus*), in northeast India and suggests the nomen *Polypedates teraiensis* for the northeast Indian population of *Polypedates leucomystax* species complex.

Introduction

The extant amphibian genus *Polypedates* of the family Rhacophoridae (Anura) is known to contain twenty four species of which seven species are so far recorded from the northeast India (Annandale, 1912; Chanda 1994, Roy et al. 1998, Ahmed and Dutta 2000, Ao et al. 2003, Mathew and Sen 2010).

Annandale (1912) described a new subspecies, *Rhacophorus maculatus himalayensis* from Abor hill expedition (type locality of Kobo, alt. 400 ft. asl) near Pasighat and East bank of Siang (Dihang) river (alt. 1100 ft. asl) East Siang district, Arunachal Pradesh, India which was later elevated to the species status (*Polypedates himalayensis*) [see Chanda et al. 2000]. Ahl, 1931 considered *Rhacophorus* *himalayanus* as valid nomen for the subspecies *Rhacophorus maculatus* described by Annandale, 1912.

Gorham (1974) treated the taxon as synonymous with *Polypedates leucomystax* (Gravenhorst, 1829). Dubois (1986) considered *Polypedates himalayensis* valid, but his designation of MNHN 1983.1170, from "Rakshe, 2000-2070 m, East-Nepal" as the neotype was set aside with the discovery of the syntypes by Chanda et al. (2000). Schleich and Kästle (2002) placed it in the synonymy of *P. leucomystax* and Frost

(2017) maintained this nomen as synonym to both *Polypedates leucomystax* and *Polypedates maculatus*.

Since the original descriptions, the nomen *Polypedates himalayensis* has been treated in various ways and the status of this nomen is uncertain. We evaluate the status and validity of the nomen *Polypedates himalayensis*.

Materials and Methods

Specimens were collected from different localities Assam: [Joypore RF (27°11' - 27°20' N and 95°26' - 95°29' E), Podumoni-Borjan-Bherjan WLS (27°23' - 27°28' N and 95°29' - 95°36' E), Nambor-Garampani WLS (26°23' - 26°25' N and 93°51' - 93°55' E), NP (26°55' - 27°03' N and 92°40' - 93°06' E), Garbhanga Reserve Forest (26°07' - 26°09' N and 92°33' - 91°55' E), and Mizoram: Tamdil (92°39' - 92°57' E and 23°43' - 23°58'). Further collections were also made from Orissa: [Bhubaneswar (20°16' N & 85°51' E) and Baripada (21°56' N & 86°44' E)].

Polypedates teraiensis
(lateral view)
Photo Credit: Abhijit Das

Jayanta Gogoi¹ & Saibal Sengupta²

¹Arya Vidyapeeth College, Guwahati 781016, Assam

²Assam Don Bosco University, Tapesia Sonapur, Assam - 782402, India

Corresponding author e-mail: senguptasaibal@yahoo.co.in



The collections were deposited in the Museum of Arya Vidyapeeth College (AVCM) and catalogued.

Types and Voucher specimens of *Polypedates* species of different museums AVCM, ZSIC and ZSIS) and personal collections (ID, SKD, MFA & BC) were also examined.

The specimens were measured using a Mitutoyo Dial Calipers (± 0.01 mm accuracy) to record morphometric parameters for adult and larva. Abbreviations of the parameters along with their full forms, as follows;

Adult : SVL - (Snout to vent length) from tip of snout to vent; HL - (Head length), from angle of jaw to the tip of the snout; ED - (Eye diameter), from anterior corner to posterior corner for the eye; SL - (Snout length), from anterior corner of eye to the snout tip; EN - (Eye to nostril distance), from the anterior corner of eye to center of nostril; UE - (Upper eye lid), vertically between both edges; IAE - (Inter space anterior eye), space between the anterior corner of eye; IPE - (Inter space posterior eye), space between posterior corner of eye; Pupil - Vertical/horizontal orientation of pupil; NS - (Nostril to snout distance), from the center of nostril to the tip of the snout; TE - (Tympanum to eye distance), from posterior corner of eye to anterior edge of the tympanum; HWAJ - (Head width at jaw), distance between the two lateral angles of jaw; HWPE - (Head width posterior to eye), distance between the two lateral sides at

posterior corner of eye; HWAE - (Head width anterior to eye), distance between the two lateral sides at anterior corner of eye; HWN - (Head width at nostril), distance between the two lateral side at nostril; VR - (Vomerine ridge), position, orientation, size; IVR - (Inter-vomerine ridge), space between the closest edge of both vomerine; IOS - (Inter orbital space), minimum distance between the two eyes from the base of the eyelid dorsally; INS - (Inter-narial space), distance between two nostrils at center of each nares; HTYD - (Horizontal tympanic diameter), maximum distance from the anterior to the posterior corner of the tympanum; VTYD - (Vertical tympanum diameter), maximum distance from the dorsal to the ventral corner of the tympanum; HDN - (Head depth at nostril), from dorsal surface of the nasal bone to the ventral surface of the maxilla; HDE - (Head depth anterior to eye), from dorsal surface touching anterior corner of eye to the ventral surface of the maxilla; HDAJ - (Head depth at angle of jaw), from dorsal surface of the cranium directly above angle of the jaw to the ventral surface of the maxilla; MN - (Mandible nostril), from mandible at jaw angle to centre of nostril; MAE - (Mandible anterior eye), from mandible at jaw angle to anterior corner of eye; MPE - (Mandible posterior eye), from mandible at jaw angle to posterior corner of eye; Snout - Shape from dorsal view; FLL - (Fore limb length), from arm-pit to tip of 3rd finger; Fore-arm - From posterior end of

Polypedates himalayensis shows distinct variation in body size between male and female
Photo Credit: Indraneil Das



Radio-ulna to tip of 3rd finger; Hand L - From base of first finger to tip of 3rd finger; IMC/PT - (Inner meta-carpel/ palmar tubercle), presence/absence, from base to tip of the tubercle; MMC - (Middle meta-carpel tubercle), presence/absence, from base to tip of the tubercle; OMC - (Outer meta-carpel tubercle), presence/absence, from base to tip of the tubercle; NP - (Nuptial pad), from base to tip of the pad; F1, F2, F3, F4 - From base to tip of the 1st, 2nd, 3rd, 4th finger; F1D, F2D, F3D, F4D - (Disc diameter of the 1st, 2nd, 3rd, 4th finger), from the tip of the last phalange to the tip of the disc; HLL - (Hind limb length), from anus to tip of 4th toe; TBL - (Tibia length), from knee to tibio-tarsal articulation; TBW - (Tibia width), maximum width of tibia; Ft L - (Foot length), from base to tip of the 4th toe; IMT - (Inner meta-tarsal tubercle), presence/absence, from base to tip of the tubercle; OMT - (Outer meta-tarsal tubercle), presence/absence, from base to tip of the tubercle; SAT - (Sub-articular tubercle), presence/absence, SNT - (Supernumerary tubercle), presence/absence, TTA - Tibio tarsus articulation; T1, T2, T3, T4, T5 - Toe length. (From base to tip of the 1st, 2nd, 3rd, 4th & 5th toe); T1D, T2D, T3D, T4D, T5D - (Disc diameter of the 1st, 2nd, 3rd, 4th & 5th toe), from the tip of the last phalange to the tip of the disc; Toe web formula has been portrayed following Savage & Heyer (1997).

Tadpole: The eggs laid in foam nest in the specially designed enclosures were allowed to develop naturally and the larval stages were collected for taxonomic characterization. Grosjean (2005) pointed out that a development climax for most characters is reached by the tadpoles from 32 - 40 Gosner (1960) stages and as we observed that this climax is reached at Gosner stage 36 in *Polypedates himalayensis*, we selected larval stage 36 in the present study. The following larval parameters were measured.

SVL- (snout vent length), from tip of snout to vent tube; BL- (Body length), from tip of snout to body-tail junction; BW- (Body width), maximum width of the body; BH- (Body height), maximum height of the body; HL- (Head length), distance from tip of snout to posterior margin of eye; HD- (Head depth), maximum height of head; ODL- Oral



Foam nest constructed on ground by *Polypedates himalayensis*

disc length; ODW- Oral disc width; EN- (Eye to nostril distance), distance between anterior point of eye and middle nostril; NS- (Nostril to snout distance), distance between anterior point of nostril to tip of the snout; INS- (Inter-narial space), distance between nostrils; IOS- (Inter-orbital space), minimum distance between the eyes; ED- (Eye diameter), distance between anterior corner of eye to the posterior corner of the eye; SPL- Spiracle length; TL- (Tail length), from the body-tail junction to the tip of the tail; TMW- (Tail maximum width), maximum thickness of the tail at mid-tail position; MTH- (Maximum tail height), greatest distance between dorsal and ventral fin margin; BS - Beak serration; LTRF - (Labial tooth row formula), number and status representation of the larval Keratodont. The keratodonts proximal to the upper beak were termed as 'A' and counted starting from the outer fringe of oral armature, while those distal to the lower beak were termed as 'P' and counted starting from near lower beak.

The advertisement calls were recorded using an analogue device, Sony DSC micro cassette recorder coupled with a unidirectional microphone, Ahuja XLR 1000. Calls were digitalized into WAV formats after filtering the disturbances using Computer software "Goldwave 5.55" and "Audacity 1.2.6". The filtered calls were run in the "Sound Ruler, Gnomovision version 69" software to record various acoustic parameters following Roy (1993) and Matsui (1994).



Abbreviation used

RF = Reserve Forest, WLS = Wild life sanctuary, NP = National park, AVCM = Museum of Arya Vidyapeeth College, ZSIC = Zoological Society of India, Kolkata, ZSIS = Zoological Survey of India, Eastern Regional Station, Shillong, ID = Indraneil Das, SKD= Sushil Kumar Dutta, MFA= Md. Firoz Ahmed, BC = Basundhara Chetri

Results and discussion

The *Polypedates himalayensis* is described from the syntype specimen and also from the collections made during the present study

Adult

Original description of Annadale

(1912): "A well-developed parietal-squamosal arch; dorsal surface of skull smooth; skin of dorsal surface of head free". Distributed in Eastern Himalayas, Assam, Western China.

Description from syntype (ZSIC 16944) (Table I) [ZSIC 16969 has been mutilated and could not be measured].

A moderate size species. Head slightly longer than broad, dorsally flat to slightly concave and triangular; head length more than three times the head depth at nostril, about 1/3rd of the snout-vent length. The cephalic skin free from the nasal and the fronto-parietal. Canthus rostralis oblique;

loreal slightly concave. Snout obtusely pointed and project beyond the lower lip, little less than half of the head length, about 18% of the snout-vent length. Nostril closer to the snout than eye; eye-nostril (EN) more than 63% of the snout length, about 1/3rd of the head length. Eye large, its diameter about 40% of the head length, more than 3/4th of the snout length, about 15% of the body length, more than the distance from eye to nostril.

Nostril oval without flap laterally. Internarial space smaller than the inter-orbital space; interorbital space slightly convex, greater than upper eye lid. A symphyseal knob on mid lower jaw. Tympanum roughly rounded, smaller than eye, separated from eyes by a space of about a quarter of the tympanum size.

Hand with inner metacarpal tubercle, middle metacarpal tubercle and outer metacarpal tubercle. Fingers long, tips with well-developed circular discs and provided with terminal knuckle, circum-marginal groove and basal groove. The relative finger length - $F3 > F4 > F2 > F1$; sub articular tubercles small, not well developed; super numerary tubercles present. Rudimentary web between the 1st- 2nd and, 2nd - 3rd fingers.

Tibia long, more than half of the body length, about six times of its width. An elongated inner metatarsal tubercle (IMT); outer metatarsal tubercle absent. Tip of toes

Polypedates leucomystax
Female, AMNH 68169 from
Abuyog, Philippines.
Photo Credit: Abhijit Das

dilated into disc, disc with terminal knuckle, basal groove and circum-marginal groove. Tibio tarsal articulation reach eye. Relative toe length - $T4 > T5 > T3 > T2 > T1$; Toe webbing - $I1 - I10 - 2III1/2 - 2IV11/2 - 1/2V$.

Secondary Sexual Characters (male): nuptial pad on 1st and 2nd fingers, elongated paired internal vocal sacs at the base of jaw angle.

Description based on collections from NE India (Table I): A medium sized. Head length almost equal to its width, slightly convex dorsally; head length more than three times head depth at nostril, about 1/3rd of the snout-vent length. Skin of head free from the nasal and the fronto-parietal bones. Canthus rostralis rounded; loreal almost vertical and concave. Snout obtusely pointed, more than half of the head, about 1/5th of the body length. Nostril closer to the snout than eye, eye-nostril space more than 61% of the snout length and about 1/3rd of the head length. Eye moderate, about 35% of the head length, its diameter lesser than 3/4th of the snout length, slightly greater than the distance from eye to nostril. Nostril oval without flap laterally. Inter narial space smaller than the interorbital space. Pineal body absent. Symphysial knob on mid lower jaw present. Tympanum rounded, smaller than eye, about 60% of the eye diameter, separated from the eye by a distance lesser than horizontal tympanic diameter.

Fore limbs are moderately long, more than 1½ of the hand length. Hand provided with inner metacarpal tubercle, middle metacarpal tubercle and outer metacarpal tubercle. Inner metacarpal tubercle about 1/5th of the hand length. Fingers long, tips of fingers with well-developed circular discs having terminal knuckle, circumferential groove and basal groove. The relative finger length - $F3 > F4 > F2 > F1$; relative finger disc size - $F3D > F4D > F2D > F1D$. Subarticular tubercle prominent. Fingers with rudimentary web at the base of the 1st - 2nd and, 2nd - 3rd fingers. super numerary tubercles present.

Hind limb long, tibia long, 30% of the leg length, about half of the body length, about four to five times more than its width. Inner metatarsal tubercle elongated and about 10% of the foot length; outer metatarsal tubercle absent. The tip of toes dilated into

disc having terminal knuckle, basal groove and circumferential groove. Tibio tarsal articulation reach the eyes. Relative toe length - $T4 > T5 > T3 > T2 > T1$; relative toe disc size - $T4D > T5D > T3D > T2D > T1D$; Toe webbing- $I1\frac{1}{2} - I11\frac{1}{2} - 2III\frac{1}{2} - 2IV1\frac{1}{2} - \frac{1}{2}V$. Vomerine ridge narrow, elongated, positioned between choanae, angular to the body axis. Choanae oval.

Dorsum and upper flank golden-brown to coffee-brown with scattered black spots, occasionally with blackish unpatterned blotches; two dorso-lateral broken but distinct lines from inter orbital position to groin present. Upper lip brownish bordered with a faint white line; lower lip brown. Circular tympanum flesh pink to translucent dark tan. Limbs and digits with oblique cross bars; inner and outer thigh reticulated with dark brown stripes forming white oval to polygonal blotches. Dorsal surface mostly smooth with strong granulation around vent; ventral strongly granulated; ventrally lower lips, tibia and tarsus smooth. A dorso-lateral sharp supra tympanic fold extend from posterior eye to armpit present. A dark brown streak extend just beneath the supra-tympanic fold from posterior tympanum to mid-flank, occasionally up to the groin. Belly creamy white, throat, thigh and chest white with brown speckles; Ventral thigh light reddish. Toe web uniformly brown.

Sexual characters: Male- internal vocal sac at base of mouth, nuptial pad at the base of 1st and 2nd fingers.

Female: distinctly larger than the male.

Ecological notes: This species was observed only during April to June, which is also the breeding period. This was found to inhabit damp ground covered by undergrowths or leaf litter in the open areas of forests, without crown canopy but surrounded by trees. Calling males were always traced below leaf-litter or undergrowths or sometimes fallen logs. Calls started with low pitched single interval clucks and ending with fast repetition of clucks that occasionally lasted for 60 to 90 seconds. Eggs were observed to lay in foam nest constructed under leaf litter or ground vegetation.

Tadpole

Original description of Annadale (1912)

Head and body moderately flat above, ovoid, rounded in front, convex on ventral surface.

Mouth nearly terminal, comparatively small, lips relatively narrow, both directed forward; upper lip smooth except at the corners, which bears numerous rounded papillae; lower lip with a fringe interrupted in the middle, and consisting of similar papillae about three deep;

LTRF I:3+3/I+I:2 or I:3+3/3; beak in two parts; the upper beak not hooked, the lower crescentric; both parts massive, both serrated.

Eyes and nostril- Eyes lateral directed outwards; nostril nearer tip of snout than eye.

Glands- A large gland in front of and slightly below each eye. Spiracle sinistral, pointing backwards and little upwards, flap like, large. Anus dextral. Tail long and slender, twice as long as head and body, sharply pointed; its outline not strongly

sinuous; fin membranes deep through out its length.

Colouration - Mottled with dark brown on dorsal surface and sides; fin membranes minutely spotted; ventral surface white.

Description based on collections from NE India (Table III)

Larva moderate [mean total length (TL):30.692 mm]. Snout-vent length constituting 1/3 rd of total length; head large; Body oval and elongated in dorsal view; ovoid in lateral view; widest at the middle of the intestinal coil, body width more than half of Snout-vent length; body height marginally less than width. Snout rounded in dorsal view and maximum width immediately behind eyes. Eyes moderate size, larger than the space between nostril and snout. Positioned more dorsally than dorso-laterally and directed laterally. Inter orbital space about 3/4th of body width. Nares elliptical, small with a mid-dorsal bifid skin projection; positioned more dorsally than dorsolaterally; directed antero-laterally; the internarial almost half of interorbital space.

Table I: Relative measurements of *Polypedates himalayensis* comb nov., *Polypedates leucomystax* (Borneo) and *Polypedates teraiensis* (NE India)

Variable	<i>Polypedates himalayensis</i>			<i>Polypedates leucomystax</i> (Borneo)	<i>Polypedates teraiensis</i> (NE India)	
	Syntype ZSIC 16944	Male (24) Mean + SD	Female (21) Mean + SD	Male (2)	Male (23) Mean + SD	Female (22) Mean + SD
SVL	31.84	44.948+ 3.32	60.357+3.07	47.17+1.23	53.79+ 2.26	70.687+7.35
HL:SVL	0.366	0.3228+0.003	0.300+0.002	0.320+0.014	0.322+0.009	0.300+0.012
HL:HW	1.074	1.0213+0.008	0.92561+0.031	1.105+0.007	1.009+0.006	1.009+0.027
SL:HL	0.494	0.53894+0.015	0.53244+0.029	0.505+0.035	0.532+0.021	0.517+0.010
SL:SVL	0.181	0.17489+0.004	0.19321+0.039	0.165+0.007	0.171+0.003	0.157+0.005
EN:HL	0.314	0.336+0.007	0.337+0.008	0.335+0.021	0.359+0.012	0.354+0.015
EN:NS	2.10	1.908+0.048	1.664+0.051	1.785+0.007	2.089+0.066	2.511+0.071
EN:SL	0.636	0.6167+0.004	0.6371+0.052	0.665+0.007	0.617+ .025	0.637+0.027
ED:SVL	0.145	0.1159 +0.003	0.10833+0.008	0.115+0.007	0.107+0.002	0.099+0.006
ED:SL	0.801	0.658+0.022	0.66183+0.028	0.710+0.099	0.619+0.016	0.656+0.029
ED:EN	1.259	1.0664+0.034	1.046+0.644	1.060+0.141	0.921+0.028	0.931+0.055
ED:HL	0.396	0.35933+0.012	0.3522+0.022	0.355+0.021	0.333+0.005	0.329+0.163
INS:IOS	0.838	0.785+0.025	0.684+0.043	0.715+0.007	0.616+0.002	0.592+ 0.028
IOS:UE	1.36	1.2374+0.199	1.6066+0.117	1.350+0.155	1.630+0.129	1.615+0.247
HTYD:ED	0.638	0.5762+0.139	0.63817+0.034	0.615+0.021	0.726+0.008	0.673+0.039
TBL:SVL	0.579	0.52639+0.040	0.55306+0.04	0.490+0.001	0.515+0.023	0.521+0.021
TBL:TBW	5.984	5.3782+0.061	4.256+0.075	5.580+0.014	4.941+0.036	4.681+0.313
TE:HTYD	0.251	0.42578+0.012	0.4165+ .013	0.345+0.064	0.348+0.013	0.431+ 0.052

Spiracle sinistral, slightly bulging laterally, short and narrow, opening at the middle third of body, directed postero-dorsally, at the height of the middle of the lower part of the caudal muscle; inner centripetal wall fused to the body wall and longer than the external centripetal wall; closer to end of body than to snout, and opens as an oval slit.

Tail long. Tail musculature moderate progressively tapering, marginally falling short of tail tip. Tail maximum width lesser than body width; tail height point of maximum height of tail located just before the proximal third) exceeded the body height; upper fin extending up to the posterior edge of head, both fins slightly convex; tip pointed.

Vent tube short, dextral, fused to the ventral fin, gradually tapering to narrow lateral opening; directed latero-ventrally, entirely attached to ventral fin but free from tail muscle.

Sub-dermal gland consisting of a pair of postorbital gland (semicircular aggregation) about one eye-length behind eye. Lateral line present on snout and dorsum, absent on tail.

Oral disc anteroventral, not emarginate and with marginal papillae row uniseriate ventrally and biseriate laterally; lips expanded into horizontal orientation; open mouth funnel almost as wide as body; lateral lip corners pointed, Papillae cylindrical with a rounded tip, increasing in length anteroposteriorly, of moderate size except the marginal papillae of the posterior part of the lower labium moderately long. No denticulate papillae, anterior labium not separate from posterior labium, marginal papillae of anterior labium confined to lateral corners, posterior labium with continuous row of marginal papillae.

Upper lip not as deep (anteroposteriorly) as lower, separated from snout by marginal groove.

Beaks thin, both keratinised and serrated along outer edges with many long, fine, pointed serrate, upper with median notch.

Upper jaw sheath "arc" shaped and narrower than the lower one, lower jaw sheath 'V'-shaped.

LTRF 1:3+3/3. A1 almost touching lateral



papillae, A2 touching beak dorsally, A2, A3 & A4 progressively shortened; P1 and P2 almost equal while P3 shorter.

In various literatures occurrence of *Polypedates leucomystax* has been reported from various parts of northeast India along with the presence of *Polypedates teraiensis*, the nomen which has been proposed for *P. leucomystax* for the population in Nepal, Bangladesh and India. In the present study, the validity of this complex was also investigated.

Original description of *Polypedates leucomystax* by Gravenhorst (1829):

"Toes half webbed, abdomen and femur granulated below, rusty in colour above, margin of the upper jaw and lateral border of the anterior feet with white" [translated]

Description based on material examined during the present study

[1.a] Bornean population: - The present description is based on two male specimens from personal collection (ID). A medium sized frog, head longer little than broad, dorsally flat to slightly concave and triangular; head length more than four times the head depth at nostril, head length about 1/3rd of the snout-vent length. The species have much of the head skin co-ossified with the nasal and the fronto-parietal bones. Canthus rostralis oblique and loreal region slightly concave. Snout obtusely pointed, project beyond the lower lip more than half of the head length and lesserer than 17% of the snout-vent length. Nostril closer to the snout than eye, eye nostril distance little lesserer than two times the distance between nostril and snout, eye-

Foam nests of *Polypedates teraiensis*

nostril distance more than 65% of the snout length and about 1/3rd of the head length. Eye diameter about 33% of the head length, 3/5th of the snout length, about 10% of the body length and slightly lesser than the distance from eye to nostril.

Nostril oval without flap laterally. Internarial space less than the inter-orbital space, interorbital space slightly convex. Pineal ocellus obscure. Symphysial knob on mid lower jaw. Tympanum round, smaller than eye, separated from eyes by a space of lesser than half of the tympanum. Choanae oval. Vomerine ridge oblique, angular to the body axis, originating from the anterior corner of choanae and extends beyond the lower edge.

Fore limb more than 1½ of the hand length. Hand provided with inner metacarpal tubercle, middle metacarpal tubercle and outer metacarpal tubercle. Inner metacarpal tubercle less than 1/5th of the hand length. Fingers long, tips of fingers with well developed circular discs having terminal knuckle, circum-marginal groove and basal groove. The relative finger length - $F3 > F4 > F2 > F1$; relative finger disc size - $F3D > F4D > F2D > F1D$. SAT small and not well developed; Super numerary tubercles absent. Rudimentary web between the 1st – 2nd and, 2nd – 3rd fingers. Hind limb long, more than 11/2 times than snout-vent length. Tibia long, 30% of the leg length, about half of the snout vent length, 51/2 more than its width. An elongated inner metatarsal tubercle (IMT). Outer metatarsal tubercle absent. Tip of toes dilated into disc, disc with terminal knuckle, basal groove and circum-marginal groove. Tibio tarsal articulation reaches beyond anterior corner of eyes or at least midway between eye and nostril. Relative toe length - $T4 > T5 > T3 > T2 > T1$; relative toe disc size - $T4D > T5D > T3D > T2D > T1D$. Toes webbing - $I1-1\frac{1}{2}II1-2III1-2IV2-1V$ [Table I].

Dorsum grey to golden brown and with four distinct dark dorsal stripes, lateral two originate from posterior corner of eye while the middle two originate from snout, all four gradually disintegrate into brown spots towards the posterior proximities. Upper part of flank light brown while lower flank and groin reticulated with brownish streaks forming oval spots in between. Upper lip



Amplexus in *Polypedates teraiensis*

pale white and lower lip brownish. Fore limb, thigh, tibia and tarsus with coffee brown transverse bands with occasional specks of white on the thighs. Inner thigh marbled with oval brownish spots, outer thigh reticulated. Skin of snout, inter orbital space, lateral side of head, upper eyelids rough with rounded tubercles. Ventrally white; strong granulation on the ventral aspect of the throat, chest, belly and thigh, smooth on tibia and tarsus. A supra-tympanic fold extend dorso-laterally from posterior corner of eye to the armpit, ending abruptly and not converging to armpit.

Sexual character (male): Nuptial pad on 1st and 2nd fingers, antero-posteriorly elongated paired internal vocal sacs at the base of jaw angle.

[1.b] NE Indian population: A relatively large species. Head little longer than broad, dorsally flat to slightly concave and triangular; head length more than four times the head depth at nostril, length of the head about 1/3rd of the snout-vent length. The species characterized by co-ossified skin on nasal and fronto-parietal. Canthus rostralis oblique and loreal region slightly concave. Snout obtusely pointed and projects beyond the lower lip, more than half of the head length and about 17% of the snout-vent length. Nostril much closer to the snout than eye, eye-nostril more than 61% of the snout length, about 1/3rd of the head length. Eye diameter about 33% of the head length and eye diameter 3/5th of the snout length, about 10% of the body length, slightly less than the distance from eye to nostril.

Nostril oval without lateral flap. Internarial space lesser than the inter-orbital space and interorbital space slightly convex, greater than upper eye lid.

Pineal ocellus obscure. Symphysial knob on mid lower jaw present. Tympanum round, smaller than eye, separated from eyes by a space of lesser than half of the tympanum. Choanae oval. Vomerine ridge oblique, angular to the body axis, originating from the anterior corner of choanae, extend beyond the lower edge.

Fore limbs moderately long, more than $1\frac{1}{2}$ of the hand length. Hand provided with inner metacarpal tubercle, middle metacarpal tubercle and outer metacarpal tubercle. Inner metacarpal tubercle less than $\frac{1}{5}$ th of the hand length. Fingers long, tips of fingers with well-developed circular discs having terminal knuckle, circum-marginal groove and basal groove. The relative finger length - $F3 > F4 > F2 > F1$; relative finger disc size - $F3D > F4D > F2D > F1D$. SAT small, not well developed; Super numerary tubercles absent.

Rudimentary web between the 1st – 2nd and, 2nd – 3rd fingers. Hind limb long, more than $1\frac{1}{2}$ times than snout-vent length. Tibia long, 30% of the leg length, about half of the snout vent length, more than $4\frac{1}{2}$ times its width. An elongated inner metatarsal tubercle (IMT), lesser than 10% of the foot length; outer metatarsal tubercle absent. Tip of toes dilated into disc, disc with terminal knuckle, basal groove and circum-marginal groove. Tibio tarsal articulation reach beyond anterior corner of eyes or at least midway between eye and nostril. Relative toe length - $T4 > T5 > T3 > T2 > T1$; relative toe disc size $T4D > T5D > T3D > T2D > T1D$. Toewebbing - $I1-1\frac{1}{2}II1-2III1-2IV2-1V$ (Table I)

Mathew and Sen (2009) described two new species namely *Polypedates assamensis* and *Polypedates subansiriensis* from this region. However, both the species were described based on single specimen. Both the species have loose cephalic skin, head broader than long, pineal body as tiny spot and TTA reaching the nostril.

Polypedates leucomystax complex of NE India (PL NEI) was compared with the

Polypedates leucomystax (Borneo) and found to differ from the later by having larger size longer snout, smaller eye. Further, in PL NEI the tibia was longer, the TTA reached the anterior corner of the eye vs to the snout tip in *P. leucomystax*. Dubois (1986) observed relatively small SVL in Borneo population (SVL > 48.2 mm) of *P. leucomystax* than the population of Nepal terai (SVL = $51.5 - 82.8$ mm) and also relatively long tibia (TBL:SVL = $0.469 - 0.607$ in Borneo population and TBL:SVL = $0.461 - 0.532$ in Nepal population) and proposed Nepal and NE India population as a distinct subspecies of *P. leucomystax* and provided the subspecies name as *P. leucomystax teraiensis*; the Bornean population was maintained by him as nominative subspecies, *P. leucomystax leucomystax*. However he described the population of Bangladesh, Manipur, Myanmar as a transition state between these two but maintained the nomen *P. leucomystax teraiensis* for these populations. The present study supports the view of Dubois (1986).

Polypedates himalayensis differed from *Polypedates teraiensis* in having loose cephalic skin, smaller size, larger snout length, larger eye size, larger eye to nostril space, and longer hind limb; from *Polypedates maculatus* in having smaller size, larger eye diameter and longer tibia; from *Polypedates taeniatus* by showing smaller size, larger eye, longer tibia and smaller eye nostril space; from *Polypedates megacephalus* in having shorter tibia; from *Polepedates himalayensis* in having smaller size, smaller snout (length) and longer tibia; from *Polypedates subansiriensis* in having smaller size, broader head and larger eye

The larval characters of *Polypedates himalayensis* also exhibited difference from *Polypedates teraiensis*, and *P. megacephalus*. *Polypedates himalayensis* differed from *Polypedates teraiensis* tadpole in higher total length and SVL, lesser comparative inter orbital space, greater comparative inter narial space, lesser comparative tail muscle width; from *Polypedates maculatus* in having lesser body length and SVL, lesser comparative body width, greater comparative eye diameter, greater comparative inter orbital space, lesser comparative inter narial space, much smaller body width and body height, and from tadpole of *Polypedates megacephalus* in having

sinistral spiracle and LTRF I:3+3/I:I:2 or I:3+3/3

The acoustic analysis of *Polypedates* species (Table 3) is presented as below

Acoustic characters (Table III)

The call of *Polypedates himalayensis* found to include 6 to 7 notes of 5 to 18 pulses with a call duration of 4700 ms. The mean pulse peak was 3.654 ms (± 1.276) and relative pulse peak noted was 0.799 Hz ($\pm .074$).The Pulse Dominant frequency was 0.252 Hz (± 0.103)and the fundamental frequency ranged from 1139 Hz to 2211 Hz (mean 1296 ± 370). The interval between subsequent pulses in 6 pulsed note was 2.99 ms and that of 7 pulsed note was 31.96 ms with a mean pulse interval of 10.23 ms (± 11.62).

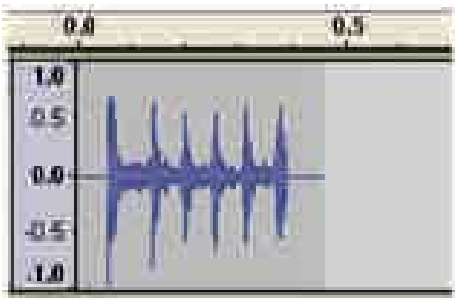


Figure 1a: WAV format of a single call of *Polypedates himalayensis*



Figure 1b: Plot spectrum at 512 frequency bin of *Polypedates himalayensis*

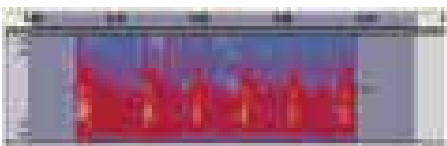


Figure 1c: Band spectrum of a single call of *Polypedates himalayensis*

The Bornean population of *Polypedates leucomystax* had a single note call which include 11 or 12 pulses with mean call duration of 1700 ms. The mean pulse peak was noted at 0.79 Hz (± 0.094) and relative pulse peak at 0.854 Hz ($\pm .032$). Pulse Dominant frequency was 0.202 Hz ($\pm .024$). The fundamental frequency started from 1148.4 Hz and reaches 1176 Hz (mean 1162 \pm 19.5). The interval between subsequent pulses in 11 pulsed note was 3.6 ms and that of 12 pulsed note was 3.9 ms with a mean pulse interval of 3.77ms (± 0.243)



Figure 2a: WAV format of a single call of *Polypedates leucomystax*

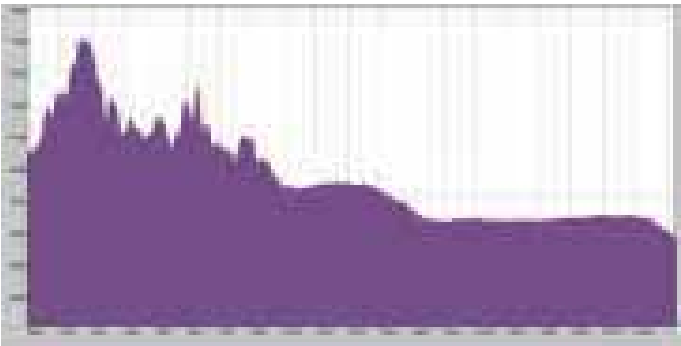


Figure 2b: Plot spectrum at 512 frequency bin of *Polypedates leucomystax*



Figure 2c: Band spectrum of a single call of *Polypedates leucomystax*

The call *Polypedates teraiensis* of N E India was with a single note of 1 to 6 pulses with mean call duration of 1750 ms. The mean pulse peak was 0.234 ms (± 0.082) and relative pulse peak recorded at 0.773 Hz (± 0.118). The Pulse Dominant frequency was 0.187 (± 0.270). The fundamental frequency started from 990.5 Hz and reaches 1205.9 Hz (mean 1051.1 ± 66.9). The interval between subsequent pulses in 1 pulsed note was -1 ms and that of 6 pulsed note was

7.096 ms with a mean pulse interval of 1.321ms (± 2.458)

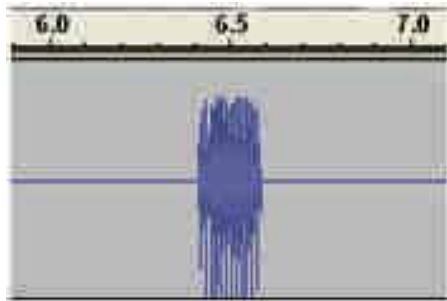


Figure 3a: WAV format of a single call of *Polypedates teraiensis*



Figure 3b: Plot spectrum at 512 frequency bin of *Polypedates teraiensis*



Figure 3c: Band spectrum of a single call of *Polypedates teraiensis*

Polypedates maculatus from Orissa was found to produce two types of calls type A and type B. Call type B was more frequently encountered with (Plate IV and V).

Call type A: Call type A was a multi note call consisting of 3 to 7 pulses with mean call duration of 114 ms. The pulse peak was 0.869 ms ($\pm .070$) and relative pulse peak was 0.788 Hz (± 0.021). The Pulse Dominant frequency was 0.336 Hz (± 0.120). The fundamental frequency started from 508.2 Hz and reached the crest of 703.4 Hz (mean 608.3 ± 76.1). The interval between subsequent pulses in 3 pulsed note was 8.46 ms and that of 7 pulsed note was 22.74 ms with a mean pulse interval of 14.61 ms (± 4.63).

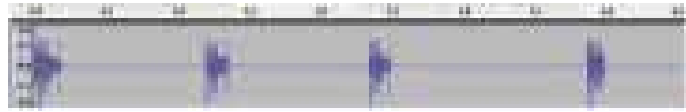


Figure 4a: WAV format of four consecutive calls of Type A

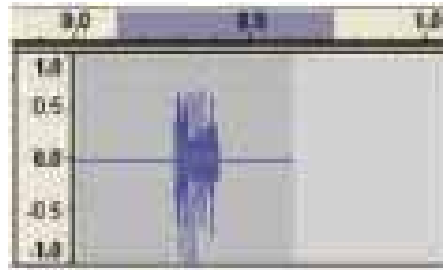


Figure 4b: WAV format of single call of type A

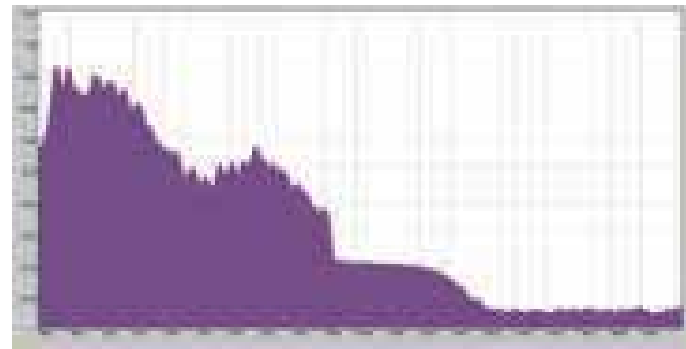


Figure 4c: Plot spectrum at 512 frequency bin of type A

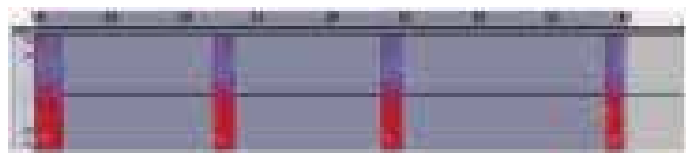


Figure 4d: Plot spectrum at 512 frequency bin of type A

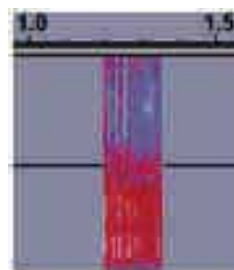


Figure 4e: spectrum of single call of type A

Call type B: This call was multi (9) note and consisted of 5 pulses each with mean call duration of 6500 ms. The mean pulse peak and relative pulse peak recorded were 0.557 Hz (± 557) and 0.82 Hz (± 0.15) respectively. The Pulse Dominant frequency was 1780.08 Hz (± 797.67). The

fundamental frequency started from 388 Hz and reached 1249 Hz (mean 663 ± 341). The interval between subsequent pulses range was between -1ms and 20.70 ms with mean interval of 14.33 ms (± 8.88).

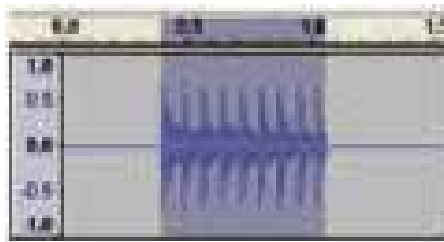


Figure 5a: WAV format of single call of Type B



Figure 5b: Plot spectrum at 512 frequency bin of Type B

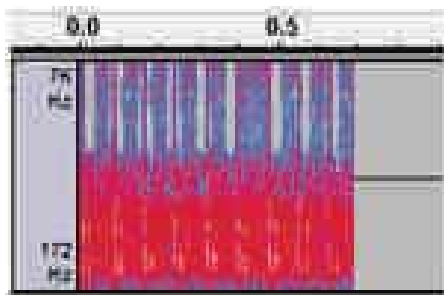


Figure 5c: Band spectrum of single call of Type B

The advertisement call analysis also suggested distinct status of *Polypedates himalayensis*. Further, it has been demonstrated that the Northeast population and Bornean population of *Polypedates leucomystax* represent two distinct populations.

The above discussion suggests a distinct specific status of *Polypedates himalayensis* and we suggest removal of nomen *Polypedates himalayensis* from the synonymy of *Polypedates leucomystax* and *Polypedates maculatus*.

Dubois (1986), considering the nature of

cephalic skin, marking on the dorsum and presence of nuptial pad on fingers, suggested the assemblage of *Polypedates* into two species groups namely- *P. leucomystax* (*P. leucomystax*, *P. megacephalus* and *P. teraiensis*) and *P. maculatus*, *P. himalayensis* and *P. zed*). In the present study all the species of both the groups were examined except for *P. zed* which was reported only from Nepal. *P. leucomystax* group has fused cephalic skin and nuptial pad on the first two fingers, besides longitudinal markings on the dorsum (Dubois 1986). In the present study besides these three features mentioned above, it was observed that the head gape size was higher in *P. leucomystax* group than that of the *P. maculatus* group and may consider as a key character that separates the members into two distinct groups.

P. himalayensis has loose cephalic skin like *P. maculatus* but contained nuptial pad on the first two fingers like *P. leucomystax*. Thus placement of this species in either of the Dubois's group became difficult and the present study suggests an intermediate group for this species. Further, *P. megacephalus*, which has been reported from Nagaland, (Ao et al. 2003) and Manipur (Ningombam and Bordoloi 2007) in northeast India, was resurrected from the synonymy of *Polypedates leucomystax* (Matsui et al 1986) and included under *P. leucomystax* group (Dubois, 1986). This species in the present study was also recorded from Sikkim. Due to the presence of non co-ossified cephalic skin but nuptial pad on both first and second fingers, this species also to be included under intermediate group.

Annandale (1912) reported *Polypedates maculatus* from Arunachal Pradesh and Roy et al. (1998) and Mathew and Sen (2010) from Meghalaya. We failed to find this species in either of the locations and could not trace any voucher specimens collected by any of the authors. Further, voucher specimens ZSIC A1684 (Collector- Pramod Goswami, in Oct 1943 from Nalabri) and ZSIC A1588 (Collector Kar Bahadur, 30.05.1960 from Cherrapunjee) have been misidentified and labeled as *P. maculatus* but in fact that are *P. himalayensis*. Thus it appeared that the reports of *P. maculatus* from northeast India was erroneous and the present study suggests removal of the species from the faunal list of northeast India.

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Materials examined:

***Polypedates leucomystax*: ID MOS0041, ID MOS0090, Borneo**

***Polypedates teraiensis*:** 8575 (ZSIC), V/A/ERS/567 (ZSIS), V/A/ERS/ZSI/766 (ZSIS),

V/A/ERS/428 (ZSIS), AVCM J-012, AVCM J-018, AVCM J-022, AVCM J-023, AVCM J-024, AVCM J-027 (Joypore, Assam), AVCM J-028, AVCM A-0032, AVCM J-033 (Podumoni), AVCM J-034 Podumoni., AVCM J-035 (Amsang R.F), AVCM J-036(Amsang R.F), AVCM J-038, AVCM J-040, AVCM J-041, AVCM J-042, AVCM J-043, AVCM J-045, AVCM J-046, AVCM J-047, AVCM J-048, AVCM J-049, AVCM J-050 (Podumoni, Assam) AVCM J-054, AVCM A-0078 (Pakke, Arunachal Pradesh), AVCM A-0087(Pakke, Arunachal Pradesh), AVCM A-0088, AVCM A-0089(Pakke, Arunachal Pradesh), AVCM A-0141, AVCM A-0182 (Pakke, Arunachal Pradesh) AVCM A-0248(Pakke, Arunachal Pradesh), AVCM A-0255, AVCM A-0141(Nambor-Garampani WLS), AVCM HT 57, AVCM HT 58, AVCM HT 60 (Kolasib, Mizoram), MFA 10017, MFA 10294 (Cheerapunjee, Meghalaya).

***Polypedates himalayensis*:** ZSIC-16944, ZSIC-16969, ZSIC-17792 & ZSIC-16958 (Abor Hill); AVCM J-001, AVCMJ-002, AVCMJ-003, AVCMJ-004 AVCMJ-006, AVCMJ-007, AVCMJ-008, AVCMJ-009, AVCMJ-010, AVCMJ-013, AVCMJ-014, AVCMJ-015, AVCMJ-016, AVCMJ-017, AVCMJ-021, AVCMJ-025 (Joypore) RE, AVCMJ-019, AVCMJ-030, AVCMJ-029, AVCMJ-020 (Podumoni WLS), AVCMJ-026, AVCMJ-031, AVCMJ-032, AVCMJ-036, AVCMJ-037, AVCMA-315, AVCMA-318, AVCMA-320, AVCMJ-018 (Pakke, Arunachal Pradesh) AVCM-SP01, AVCM-SP02, AVCM-SP03, AVCM-SP53 (Meghalaya), AVCM-HT 0002, AVCM-HT 0013, AVCM-HT 51 (Mizoram), AVCM-NL02, AVCM-NL03 (Nagaland), ZSIC-15715, ZSIC-A1684, ZSIC-A3457, ZSIC-A4416, MFA10058, MFA10060, MFA10234 (Cherrapunjee)

Table II: Measurements of tadpole (Gosner stage 36) of *Polypedates himalayensis* (n=10)

Variable	Mean +SD	Range
TL	26.15+0.03	26.10 - 26.19
SVL	9.24+0.03	9.20 - 9.28
HL	7.07+0.01	7.05 - 7.09
Tail	20.40+0.05	20.32 - 20.47
BW	5.05+0.01	5.04 - 5.06
BH	5.04+0.05	4.96 - 5.10
TMW	2.34+0.08	2.23 - 2.46
EN	1.99+0.02	1.96 - 2.02
NS	1.17+0.04	1.10 - 1.22
INS	1.61+0.17	1.42 - 1.89
IOS	3.81+0.02	3.79 - 3.84
ED	1.55+0.06	1.44 - 1.60
TH	6.14+0.08	6.00 - 6.23
BW:SVL	0.526 0.020	0.502 - 0.555

Variable	Mean +SD	Range
BH:BW	0.985 0.046	0.917 - 1.040
ED:SVL	0.160 0.001	0.159 - 0.160
IOS:BW	0.717 0.017	0.692 - 0.738
ED:NS	1.296 0.139	1.152 - 1.509
INS:IOS	0.499 0.013	0.474 - 0.510
TH:BH	1.180 0.004	1.175 - 1.185
TMW:BW	0.445 0.027	0.419 - 0.479
SVL:TL	0.339 0.001	0.337 - 0.341
HL:SVL	0.951 0.272	0.694 - 1.201
HL:TL	0.322 0.091	0.237 - 0.407
TAL:TL	0.753 0.033	0.750 - 0.758

Table III: Call features of *Polypedates* species

Species	<i>P. leucomystax</i>	<i>P. himalayensis</i>			<i>P. maculatus</i>	
Location		Borneo	northeast	northeast	Call type A	Call Type B
Call duration		1700 ms	1750 ms	4700 ms	1140 ms	6500 ms
Call peak time	Mean	47.36	0.37	1.977	0.03869	0.057
	Std. Deviation	2.23	0.17	2.378	0.01645	0
Total pulses	Mean	11.768	3.11	8.14	5.333	5
	Std. Deviation	0.445	1.9	4.55	1.366	0
Fft length	Mean	256	256	256	256	256
	Std. Deviation	0	0	0	0	0
Pulse peak	Mean	0.79	0.23	3.654	0.869	0.56
	Std. Deviation	0.0949	0.08	1.276	0.07	0.56
Pulse interval	Mean	3.775	1.32	10.23	14.61	14.33
	Std. Deviation	0.243	2.46	11.62	4.63	8.88
Peak dominant frequency	Mean	2324.4	2024	2160.7	1226.9	1326
	Std. Deviation	38.9	417	110.8	153.9	683
Fundamental frequency	Mean	1162.2	1051.1	1296	608.3	663
	Std. Deviation	19.5	66.9	370	76.1	341
Pulse rate	Mean	0.5929	2.36	0.547	0.3022	0.27
	Std. Deviation	0.069	4.58	0.385	0.1286	0.27
Pulse dominant frequency	Mean	0.202	0.187	0.252	0.336	1289.44
	Std. Deviation	0.024	0.27	0.103	0.12	378
Relative Pulse peak	Mean	0.854	0.77	0.799	0.788	0.82
	Std. Deviation	0.032	0.12	0.074	0.021	0.15

An overview of caecilians (Amphibia: Gymnophiona) of North East India

Abstract

An overview of the caecilians of North East India (NEI) is presented. Two caecilian families are known from NEI, the widespread Asian family Ichthyophiidae, and the NEI-endemic and monotypic family Chikilidae. There are currently 12 species of caecilians reported from NEI, four species of *Chikila*, seven species of striped *Ichthyophis*, and one species of unstriped *Ichthyophis*. This overview provides the progress of research on NEI caecilian fauna to date, its diversity, taxonomy, systematics, biogeography, natural history, and distribution. Based on extensive direct interactions with local people in NEI for several years, it is clear that caecilians are generally despised due to a widespread misconception that they are venomous. The general public in NEI clearly needs a sustained community education about caecilians so that they can become more sympathetic towards them. The paper also discusses the general conservation scenario, or the lack of it, in NEI, and the plight of basic research, with an appeal for the respect that scientific merit deserves.

Introduction

Caecilians (Gymnophiona Müller, 1832) are completely limbless and girdleless snake-like amphibians. They are an ancient lineage, and are sister to all other extant amphibians (Zardoya & Meyer 2000). For almost a century the systematic placement of caecilians remained uncertain; they were mostly thought to be degenerate or naked reptiles, or eels (e.g., Linnaeus 1758, Schneider 1801, Seba 1735, Shaw 1802). Seba (1735) in his Thesaurus, published the first account dealing with caecilian species under the name 'Caecilia'. The first definitive indications of caecilians to be amphibians were the discovery of gills in juvenile caecilians by Müller (1831, 1835) and Hogg (1841). Duméril and Bibron's (1841) report on several aspects of caecilians anatomy provided more incisive evidence to support that caecilians belong to amphibians. However, debates still continued during the 19th century, but roughly by around the 1880s most workers accepted that caecilians were amphibians. The first caecilian species to be scientifically described was *Caecilia*

tentaculata by Linnaeus (1758) providing for the first time official recognition for the name 'Caecilia', and this name became the first generic name in Gymnophiona.

Caecilians are primarily adapted to a secretive burrowing lifestyle as adults, except for a South American group—the typhlonectids—which are secondarily aquatic or semi-aquatic (Taylor 1968, Wilkinson & Nussbaum 1999). Caecilians have elongated tube-like bodies with external rings all along the length of the body, each of which is called an annulus. Caecilians may or may not have tails; a true tail is characterised by the possession of vertebrae posterior to the cloacal vent, as in the family Ichthyophiidae; the tail is absent in members of the family Chikilidae (Kamei et al. 2013). Tails are considered an ancestral feature, and absence of tails as

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Amphibia,
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Teresomata

Chikila fulleri female with her
litter.

Rachunliu G. Kamei

Greenwoods Cottage, New Ministers Hill, Kohima 797 001,
Nagaland, India.

Corresponding author e-mail: kamei.chun@gmail.com



derived. Adult caecilians have protractile structures called tentacles on each side of the snout, located typically between the eyes and the nostrils, that are thought to have a sensory function (Duellman & Trueb 1994, Himstedt 1996, Noble 1931, Taylor 1968). Caecilians possess a unique, dual jaw-closing mechanism consisting of an ancestral component and a unique, novel component (Nussbaum 1977, 1983).

Caecilians have a remarkable diversity of reproductive strategies; they are reported to have oviparity with both direct and indirect development and at least two modes of viviparity (*sensu* Blackburn 1992, 2000; e.g., Kouete et al. 2012, Kupfer et al. 2006, Loader et al. 2003, Wilkinson & Nussbaum 2006).

Unlike in anurans (exception tailed frogs, *Ascaphus*) and caudates where external fertilisation is the typical mode of fertilisation, all species of caecilians practice internal fertilisation (e.g., Taylor 1968, Wake 1977) through an intromittent organ, or phallus, in males (e.g., Gower & Wilkinson 2002).

Caecilians are currently known to occur in the tropical (and some adjacent subtropical) regions of Asia, Africa, the Seychelles islands and Central and South America. To date there are 205 nominal species (Frost 2017) classified in 34 genera (Wilkinson et al. 2011). The latest family-level classification of caecilians recognises ten distinct caecilian families (Kamei et al. 2012, Wilkinson et al. 2011), each of which has an ancient (Mesozoic) origin. Most of the families are diagnosable by small sets of characters (Kamei et al. 2012, Wilkinson et al. 2011). Caecilians remain very poorly studied, in several aspects—including their lower-level taxonomy, breeding biology, life history, conservation requirements and/or status. Gower and Wilkinson (2005) highlighted the lack of even basic information for a majority of caecilian species; detailed ontogenetic studies, sexual variation, and morphological variation within and between populations of caecilians are badly wanting (Nussbaum & Wilkinson 1989). Caecilians' secretive burrowing lifestyle and the consequent difficulty to find them, requiring physical labor [skilled and dedicated soil digging, e.g., Gower & Wilkinson (2005), Malonza & Müller (2004)], is one of the biggest

challenges to the study of caecilians, and perhaps is responsible for the relative under representation of specimens in museum collections. This "rarity" of specimens has resulted in a large number of very poorly circumscribed species as early original descriptions were often based on a unique type specimen (e.g., Alcock 1904, Taylor 1968), or small sample size (two to three animals), and/or just one sex.

History of NEI caecilian studies

North East India (NEI) is the easternmost region of India comprising the contiguous seven states (Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura), Sikkim, and the northern districts (Darjiling, Kalimpong, and Jalpaiguri) of West Bengal, and shares international boundaries with Bangladesh, Bhutan, China, Myanmar and Nepal (Fig. 1).

The region is located between 29.46°–21.96°N and 97.39°–88.88°E, and forms a significant portion of two global biodiversity hotspots—the Indo-Burma and the Himalaya hotspots—ca. 8.2% of the 3,114,763 km² occupied by the two hotspots combined (Mittermeier et al. 2004) with a larger bulk of the region falling in the Indo-Burma hotspot. Aspects such as diverse physiography; vegetation diversity ranging from tropical rainforests to alpine scrubs (Mani 1974); extensive altitudinal range (from sea level to more than 8500 m asl); very heavy precipitation, being one of the highest rainfall-receiving regions in the world (Jain et al. 2013), provide unique micro-/habitats for amphibians making NEI one of India's two main hotspots (the other being the Western Ghats, Inger & Dutta [1986]) for its diversity and endemism.

Amphibians of NEI have rarely been included in regional (or global) phylogenetic and biogeography studies—e.g., on caecilians (Kamei et al. 2012), and anurans (Biju et al. 2014, 2016, Mahony et al. 2017). Prior to 2009, very little was known about NEI's caecilian diversity, taxonomy or its phylogenetic affinities. At the turn of the 20th century, the region with a geographical area of about 255,000 km² was reported to have only four poorly circumscribed caecilian species, known altogether from ten adult museum specimens, with adults



and juveniles reported from less than 15 localities (Alcock 1904, Pillai & Ravichandran 1999, Taylor 1960). The first caecilian species described from NEI was *Herpele fulleri* Alcock, 1904 from Assam. After a hiatus of nearly 50 years, a second caecilian species, *Ichthyophis sikkimensis* Taylor, 1960 was described from 'Darjeeling' and 'Sikkim'. Pillai and Ravichandran (1999) in their taxonomic study and review of the Indian caecilians then known added two new species, *Ichthyophis garoensis* and *I. husaini* from Meghalaya. A few subsequent publications on NEI amphibians included reports of the occurrence of *I. garoensis* from additional localities (e.g., Choudhury et al. 2002, Mathew & Sen 2009). The original descriptions of all four of these NEI caecilians were sketchy, based on a small sample size of one to four specimens, were incomplete, and information on intraspecific variation was severely lacking. Caecilian studies based on contemporary field

research remained neglected for about a decade after Pillai and Ravichandran's (1999) work.

Kamei et al. (2009) conducted the first expansive field-based caecilian research, covering all NEI states (bar Mizoram, see Kamei et al. 2013), and based on their new collections they described three new species in the family Ichthyophiidae: *Ichthyophis khumhzi*, *I. moustakius* and *I. sendenyu*, from Manipur and Nagaland. Shortly after, Mathew & Sen (2009) described an additional three new species in the same family from Meghalaya, *Ichthyophis alfredi*, *I. daribokensis* and *I. nokrekensis*. Kamei et al. (2012) discovered an ancient, endemic, and an intriguing lineage of caecilians from NEI that was described as a new genus *Chikila* in a new family Chikilidae, and Kamei et al. (2013) described three new chikilid species: *Chikila alcocki*, *C. darlong* and *C. gaiduwani* from several NEI states.

Figure 1. Map of North East India (NEI) showing the distribution of *Ichthyophis* species in NEI. Localities of *Ichthyophis garoensis* are from Kamei & Biju (2016). Localities for all remaining species are from their original descriptions. *Ichthyophis alfredi* = blue, *I. daribokensis* and *I. nokrekensis* = orange, *I. garoensis* = green spots, *I. khumhzi* = yellow, = *I. moustakius* = turquoise, *I. sendenyu* = black, *I. sikkimensis* = pink.

Diversity, systematics
biogeography and
taxonomy

ICHTHYOPHIIDAE

Diversity: The family currently contains two genera *Ichthyophis* Fitzinger, 1826, and *Uraeotyphlus* Peters, 1880 (Wilkinson et al. 2011). The distribution of the genus *Ichthyophis* is restricted to South and Southeast Asia, while *Uraeotyphlus* is

endemic to southern peninsular India. *Ichthyophis* is the most speciose genus within the amphibian order Gymnophiona with currently 50 nominal species (Frost 2017). The species diversity of *Ichthyophis* from NEI is notable—more than 50%(eight out of 15 species) of India's *Ichthyophis* diversity is endemic to the region. The diversity is represented by one unstriped form, *Ichthyophis sikkimensis*, and seven striped forms, *I. alfredi*, *I. daribokensis*, *I. garoensis*, *I. khumhzi*, *I. moustakius*, *I. nokrekensis* and *I. sendenyu*. A second



Figure 2: Examples of habitats where caecilians can be found. Bottom image © Systematics lab.

unstriped form, *Ichthyophis husaini*, which was known from a single and faded specimen from the West Garo Hills, Meghalaya, was recently synonymised under the striped form *I. garoensis* by Kamei & Biju (2016) based on re-examination of the holotype, and comparison with new specimens from the type localities (and their vicinities) of the two species.

(ii) Systematics and biogeography: The genus *Ichthyophis* is distributed in Sri Lanka, India, eastern Nepal and southern China, and throughout Southeast Asia as far south as the Wallace's Line. Being the only caecilian group that presently occurs substantially beyond the borders of former Gondwanan landmasses, their historical biogeography has attracted attention (e.g., Duellman & Trueb 1986, Feller & Hedges 1998). Gower et al. (2002) discovered from a limited sampling that Southeast Asian ichthyophiids are monophyletic, with Sri Lankan and Western Ghats species comprising a paraphyletic grade outside this group. Gower et al.'s (2002) result is consistent with the "Out of India" dispersal hypothesis that ichthyophiids [the genus *Ichthyophis* and its sister taxon *Uraeotyphlus* (Wilkinson et al. 2011) originated in Gondwana and dispersed from South into Southeast Asia shortly after the India-Asia collision about 65–56 million years ago (Beck et al. 1995)]. The hypothesis was further confirmed by a more recent study (Nishikawa et al. 2012) that included more comprehensive Southeast Asian sampling. However, no samples from NEI have yet been included in a molecular phylogenetic analysis. Inclusion of NEI samples might provide a clearer understanding of the timing and nature of the "Out of India" dispersal. It would also help to determine whether the South to Southeast Asian dispersal route was via NEI, and if so, when the event happened. Knowledge of the phylogenetic positions of all NEI *Ichthyophis* will also establish whether one or more radiations occur in NEI, and how long these have been evolving in the region. Until such studies are done, NEI may aptly be called a 'blind spot' in the evolution of the Asian-endemic genus *Ichthyophis*.

Within India, a significant and longstanding



The inset picture shows an *Ichthyophis* among roots of grasses, which was found under a rock by the roadside.

biogeographic anomaly was recently resolved. Conflicting with the previous knowledge that *I. sikkimensis* was restricted to Sikkim and Darjeeling (northern West Bengal) (Taylor 1960, 1961, 1968), Pillai & Ravichandran (1999) assigned to this species a single specimen collected by A. F. Hutton in 1949 from Injiparai Estate, Anamalai, Kerala in the Western Ghats (see also Ravichandran 2004). Gower et al. (2017) re-examined the specimen, compared it with the type specimens of *I. sikkimensis*, and concluded based on meristic and morphometric data, and substantial differences in anterior phallodeal morphology between *I. sikkimensis* and the Western Ghats specimen, that the Western Ghats specimen is not conspecific with *I. sikkimensis*. The taxonomic conclusion that *I. sikkimensis* is absent from peninsular India has improved our understanding of biogeographical patterns in Asian caecilians.

(iii) Biology: *Ichthyophis* species in NEI have been found to be easier to locate during the heavy monsoons (beginning of June to end of July) when the animals likely migrate to near water sources such as borders of canals that feed water to paddy fields and banks of slow flowing streams for breeding (Kamei et al. 2009; Fig. 2).

Like *Ichthyophis* spp. from other regions (Breckenridge & Jayasinghe 1979, Dunker et al. 2000), those from NEI are indirect developers with an intermediate free-swimming larval stage. *Ichthyophis moustakius* is reported to lay 9–19 eggs per

clutch (n = 4 clutches, [Kamei et al. 2009]). The fragile-looking eggs are enclosed in membranes. Eggs are laid in a string (resembling pearl beads of a necklace), with consecutive eggs connected to each other by thread-like gelatinous extensions of the egg membranes. Freshly laid eggs are creamy-white and opaque (Fig. 3D).

As the embryos develop, the egg membranes turn translucent allowing one a view of the early ontogenetic development. Females are found attending their egg-clutches in underground burrows close to (seasonal or perennial) streams; marshy areas surrounding agricultural fields especially wet paddy fields; occasionally females with clutches have been found in drier habitats away from water such as under roots of herbaceous plants or near roots of banana trees. Larvae have been encountered under pebbles and leaf litter in shallow puddles of stream water. Apart from these field observations, nothing else is known about the biology of the *Ichthyophis* spp. of NEI. Although the surveys of Kamei et al. (2009) have been biased towards unprotected areas, they have been mostly found in disturbed habitats such as around wet paddy fields.

(iv) Taxonomy: The following brief taxonomic accounts of species and their distribution (Figs. 1, 4), except for Kamei et al.'s. [2012, 2013] species and *Ichthyophis garoensis*, are directly reproduced from the species original descriptions, and additional published localities when available.

Abbreviations used: AG = annular groove, CM = corner of the mouth, C1 = first collar, C2 = second collar, IM = inner mandibular (= 'splenial') tooth, L/H = total length divided by head length (the latter = distance between snout tip and nuchal groove1 directly behind CM), OM = outer mandibular (i.e., dentary) tooth, TA = tentacular aperture, TN/TE = ratio of the distances between the TA and the eye and naris, W/S = width at midbody divided by maximum width of stripe at midbody (Kamei et al. 2009, 2013; Kotharambath et al. 2012a).

Other abbreviations are: RGK (Rachunliu G Kamei), SDB (Sathyabhama Das Biju).

In the following taxonomic sections, specimens from Mathew & Sen's (2009)

were collected by Mathew & Sen and party; Kamei et al.'s (2009, 2012, 2013) specimens were collected by RGK and SDB. For historical specimens, collectors for specimens are mentioned when available.

(a) *Ichthyophis alfredi* Mathew and

Sen, 2009: This species was described based on a type series of five specimens--holotype (sex undetermined [Mathew & Sen 2009]) collected from "Meghalaya, East Garo Hills, Nokrek Biosphere Reserve, Daribokgre IB compound" and four paratypes (two adults, sexes undetermined, [Mathew & Sen 2009]; two metamorphosed juveniles)--three collected along with the holotype, one from Rongbang, 1.5 km away from Chinapat, Nokrek Biosphere Reserve (NBR), West Garo Hills.

Species diagnosis: "A fairly large species (Total length 176-330 mm) with 269-299 dorsal annuli and 262-295 ventral annuli; 5-7 annuli broken by vent, 5-7 post vent annuli; 1 dorsal transverse groove on 2nd collar; head long, depressed; tentacular aperture closer to eye than to nostril. Premaxillary-maxillary teeth c. 34-40; Prevomeropalatine teeth c. 32-40; Dentary teeth c. 32-38; Splenial teeth c. 32-40. Scales present on all annuli; lateral stripe distinct, broad, expands on collar and extend on to the lower jaw giving a clear pattern of an 'arrow shape' to gular region; body bulky, supple." (Mathew & Sen 2009).

Distribution: This species is currently known only from the type locality "Meghalaya, East Garo Hills, Nokrek Biosphere Reserve, Daribokgre IB compound" and from its vicinity in Rongbang (Mathew & Sen 2009, Fig. 1). The elevational range of *I. alfredi* is 416-1119 meters asl (Mathew & Sen 2009). See Mathew & Sen (2009) for GPS coordinates for localities.

At the type locality, *Ichthyophis alfredi* occurs in sympatry with *I. daribokensis* and *I. nokrekensis*. (Mathew & Sen 2009).

(b) *Ichthyophis daribokensis* Mathew

and Sen, 2009: This species was described based on a type series of 15 specimens--holotype (sex undetermined [Mathew & Sen 2009]) collected from "Meghalaya, West Garo Hills, Nokrek Biosphere Reserve, Rengsangre, Rongram river side" and 14

paratypes (nine adults, sexes undetermined [Mathew & Sen 2009]; five metamorphosed juveniles)--one collected along with the holotype, and one from Rengsangre, NBR, West Garo Hills; from NBR in East Garo Hills--one from Mondal Nokrat, three each from Kiwang and Sasatgre, five from Daribokgre IB compound.

Species diagnosis: "A fairly large species (Total length 155-315 mm) with 264-310 dorsal annuli and 263-304 ventral annuli; 4-7 annuli broken by vent, 4-7 post vent annuli; 1-2 dorsal transverse groove on 2nd collar; head moderately long; nuchal region moderately arched; tentacular aperture closer to eye than to nostrils. Premaxillary-maxillary teeth c. 34-40; Prevomeropalatine teeth c. 26-36; Dentary teeth c. 36-40; Splenial teeth c. 30-36. Scales present on all annuli; lateral stripe distinct, medium to broad, expand on collar, faintly extending lower jaw." (Mathew & Sen 2009).

Distribution: This species is known from multiple localities in Nokrek Biosphere Reserve (NBR) in Meghalaya--Daribokgre IB compound, East Garo Hills; Kiwang, 1 km from Daribokgre IB, East Garo Hills; Mondal Nokrat, East Garo Hills; Rengsangre, West Garo Hills; Rengsangre, Rongram river side, West Garo Hills; Sasatgre, East Garo Hills (Fig. 1). The elevational range of *I. daribokensis* is 341-1131 meters asl (Mathew & Sen 2009). See Mathew & Sen (2009) for GPS coordinates of localities.

Ichthyophis daribokensis occurs in sympatry with *I. nokrekensis* at all of the specific localities above, except Oragitok, NBR, East Garo Hills, Meghalaya, India (Mathew & Sen 2009; Fig. 1). *Ichthyophis daribokensis* occurs in sympatry with *I. alfredi* in Daribokgre IB compound, NBR, East Garo Hills, Meghalaya, India (Mathew & Sen 2009).

(c) *Ichthyophis garoensis* Pillai and Ravichandran, 1999 (Fig. 3A): This

species was described based on a holotype (an adult male, Kamei & Biju 2016) collected from "Anogiri Lake, Garo Hills, Meghalaya", India by Dr. Akhlaq Hussain, and one referred specimen (sex undetermined [Pillai & Ravichandran 1999]) collected from "Tura Garo Hills, Meghalaya", India.

Remarks: After the original description of *I.*

garoensis, the species was recorded from a few other localities (Ahmed et al. 2009, Choudhury et al. 2002, Mathew & Sen 2009). However, these subsequent publications were lacking in vital information such as, measurements or meristic data for the specimen(s), basis of determining the taxonomic identity (Ahmed et al. 2009, Choudhury et al. 2002), specific locality data (e.g., Ahmed et al. 2009), comparison with the type material (e.g., Mathew & Sen 2009), and hence did not improve the knowledge of the species. The few other publications that mention *I. garoensis* (e.g., Chanda 2002, Ravichandran 2004) provided only information directly reproduced, or based on the species' original description (Kamei & Biju 2016).

Kamei & Biju (2016) re-examined the holotype of *I. garoensis* and compared it with the holotype of a poorly circumscribed and inadequately known "unstriped" form, *I. husaini*. *Ichthyophis husaini* was described on the basis of a single badly faded (Kamei & Biju 2016) specimen that was also collected by Dr. Akhlaq Husain, only 20 km away ("The bronggiri Coffee Garden, Rongram, Garo Hills, Meghalaya") from the type locality of *I. garoensis*. The presence or absence of a lateral stripe has been used as a major taxonomic character in *Ichthyophis* taxonomy (e.g., Wilkinson et al. 2007). Pillai & Ravichandran (1999) clearly overlooked the presence of the stripe on their specimen, and thus believing it was unstriped, they named *I. husaini* without comparison with the sympatric striped *I. garoensis* (Kamei & Biju 2016). Kamei & Biju (2016), on a close re-examination of the holotype of *I. husaini* summarised that a partial stripe is unmistakable on the posterior part of the right side of the badly faded and poorly preserved specimen. Kamei & Biju (2016) revisited the differences reported by Pillai & Ravichandran (1999) between *I. garoensis* and *I. husaini* based on the holotypes and also compared their 12 newly collected specimens from nearby the two type localities. They concluded that the differences between the two types and the new specimens in the measurements, ratios, counts, or any other character are minor and can be attributed to intraspecific variation. Kamei & Biju (2016) therefore regarded *I. husaini* to represent a junior

subjective synonym of *I. garoensis*. Their taxonomic decision not only removed the uncertainty surrounding the validity of *I. husaini*, but also improved the knowledge of the species *I. garoensis* in terms of morphological variation (15 specimens) and distribution (eight localities/sites).

Species diagnosis: The species is diagnosed by having broad ($W/S < 4$) and a fairly regular, mostly solid, lateral yellow stripe extending on each side. It is not known to attain lengths greater than 325 mm. The number of AGs is fewer than 310; AGs are paler than adjacent skin. The head is somewhat more U-than V-shaped and fairly short ($26 > L/H > 21$). TAs are about twice as far from the nares than from the eyes, and are variable ($1.9 < TN/TE < 2.3$). Collars are of similar length. The numbers of IMs and OMs are similar (Kamei & Biju 2016).

Distribution: This species is known from multiple localities in Tura, West Garo Hills district, Meghalaya--"Anogini Lake, Garo Hills, Meghalaya" (Pillai & Ravichandran 1999), "The bronggini Coffee Garden, Rongram" (type locality of the *I. husaini* synonymised by Kamei & Biju [2016]), and Asanang, Barkha forest fringe, Chitotak, Tebronggre village (Kamei & Biju 2016; Fig. 1). The elevational range of *I. garoensis* is 410-530meters asl (Kamei & Biju 2017). See Kamei & Biju (2017) for GPS coordinates of localities. Localities for *I. garoensis* that are not yet verified are not included in this work.

(d) *Ichthyophis khumhzi* Kamei, Wilkinson, Gower, and Biju, 2009 (Fig. 3C): This species was described based on three specimens all collected from the type locality--holotype (an adult male) and two paratypes (both adult male) collected from "Khumhzi village (24°51'46"N, 93°37'23"E; 320 m asl), Tamenglong district, Manipur, India." (Kamei et al. 2009).

Species diagnosis: *Ichthyophis khumhzi* attains the greatest total length (greater than 400 mm) among all caecilian species described from NEI. The species is diagnosed by having narrow ($W/S > 6$) and an irregular yellow stripe laterally on each side that extends from close to CMs anteriorly to the level of vent posteriorly, not contacting the cloacal disc, and barely or

not visible on collars ventrally. The number of AGs is more than 300; AGs are darker than adjacent skin. The head is V-shaped and short ($L/H > 25$). TAs are more than twice as far from the nares than from the eyes ($TN/TE > 2$). C1 is noticeably shorter than C2. Scales are present on collars; four or five rows posteriorly on dorsum. The numbers of IMs and OMs are similar (Kamei et al. 2009).

Distribution: This species is known only the type locality in Khumhzi village, Tamenglong district, Manipur, India. (Kamei et al. 2009; Fig. 1).

(e) *Ichthyophis moustakius* Kamei, Wilkinson, Gower, and Biju, 2009 (Fig. 3B): This species was described based on a series of eight specimens--holotype (an adult female), from "Aziuram duikhun (duikhun = a pond) (25°01'43"N, 93°24'51"E; 990 m asl), Aziuram village, Tamenglong district, Manipur, India.", four paratypes (three adult females; one adult male) collected along with the holotype, and three referred specimens (two adult females, one adult male), one each from Guigailuang, Nriangluang namdaih (namdaih = a large village), Nswanram village, and Duidip Chaenglun, Bamgaizaeng village in Tamenglong district (Kamei et al. 2009).

Species diagnosis: The species is diagnosed by having broad ($W/S < 4$) and a fairly regular, mostly solid, lateral yellow stripe extending on each side from the anterior of the tail to at least the CM; the stripes are broad along mandibles with a narrow anterior gap, expanded and visible ventrally on collars, and connected to the cloacal disc by spurs. The nares and the TAs are connected by arched yellow stripes, broader at the former and narrower at the latter; the species name is derived from this character--from the Greek word *moustakius* (=moustache), referring to this distinctive (but see Kamei & Biju 2016 for variability of the 'moustache'-shaped marking) yellow, arched stripes between the TAs and nares. The species is not known to attain lengths greater than 300 mm. The number of AGs is fewer than 300; AGs are paler than adjacent skin. The head is somewhat more U-than V-shaped and fairly short ($25 > L/H > 19$). TAs are about twice as far from the nares than from the eyes, and are variable ($1.9 <$

TN/TE < 2.3). Collars are of similar lengths. Scales are absent on collars; they occur from about the fourth or fifth anterior most annulus, with five rows posteriorly on the dorsum. The numbers of IMs and OMs are similar (Kamei et al. 2009).

Distribution: This species is known only the type locality in Aziuram village, Tamenglong district, Manipur, India (Kamei et al. 2009, Fig. 1). The elevational range of *I. moustakius* is 306-1107meters asl (Kamei et al. 2009). See Kamei et al. (2009) for GPS coordinates of localities.

(f) *Ichthyophis nokrekensis* Mathew and Sen, 2009: This species was described based on a type series of 12 specimens--holotype (sex undetermined [Mathew & Sen

2009]) from "Meghalaya, West Garo Hills, Nokrek Biosphere Reserve, Sasatgre", and 11 paratypes (eight adults, sexes undetermined [Mathew & Sen 2009]; three juveniles), one collected along with the holotype, one from Rengsangre, Rongram river side, NBR, West Garo Hills; from NBR in East Garo Hills--six from Daribokgre, Forest IB compound, one each from Oragitok, Kiwang, and Mondal Nokrat.

Species diagnosis: "A fairly large species (Total length 206-325 mm) with 269-300 dorsal annuli and 266-302 ventral annuli; 4-6 annuli broken by vent, 5-7 post vent annuli; 1-2 dorsal transverse groove on 2nd collar; head moderately long; nuchal region arched; tentacular aperture closer to eye

Figure 3: *Ichthyophis* species in life. A. *Ichthyophis garoensis*, B. *I. moustakius*, C. *I. khumhzi*, D. *I. sendenyu*, a female with egg-clutch in captivity. Image 4C © Systematics Lab.



than to nostril. Premaxillary-maxillary teeth c. 38; Prevomeropalatine teeth c. 36; Dentary teeth c. 40; Splenial teeth c. 34. Scales present on all annuli; a distinct, narrow lateral stripe separates upper and lower surface from collar region to tail." (Mathew & Sen 2009).

Distribution: See distribution for *I. daribokensis* (Fig. 1) for localities and elevational range.

(g) *Ichthyophis sendenyu Kamei, Wilkinson, Gower, and Biju, 2009*(Fig. 3D): This species was described based on five specimens all collected from the type locality "Dhyutere (25°54'55"N, 94°06'19"E; 782 m asl), New Sendenyu village, Tseminyu sub-division, Kohima District, Nagaland, India." (Kamei et al. 2009) -- holotype (an adult female) and four paratypes (three adult females, one adult male).

Species diagnosis: The species is diagnosed by having broad ($W/S < 4$) and regular, mostly solid, lateral yellow stripes, one on each side extending from approximately at the level of the posterior edge of the cloacal disc to at least the eye level on the upper jaw, and midway between the TAs and the nares on the lower jaw, a broad anterior gap expanded and may/not be visible ventrally on the collars, and connected to the cloacal disc by spurs. Arched yellow stripes extend halfway from the TAs to the nares, that taper towards the nares. The species is not known to attain lengths greater than 350 mm. The number of AGs is fewer than 315; AGs are paler than adjacent skin. The head is U-shaped, and short ($L/H > 20$). TAs are less than twice as far from the nares than from the eyes ($TN/TE < 2$). Collars are of similar lengths. Scales are present in anteriormost grooves, five to eight rows posteriorly on the dorsum. The numbers of IMs and OMs are similar (Kamei et al. 2009).

Distribution: This species is known only from the type locality in New Sendenyu village, Tseminyu sub-division, Kohima district, Nagaland, India (Kamei et al. 2009, Fig. 1).

(h) *Ichthyophis sikkimensis Taylor, 1960*: This species was described based on a type series of four specimens—holotype (sex undetermined [Taylor 1960]) collected

from "Darjeeling, [West Bengal], India", and three paratypes (sexes undetermined [Taylor 1960]), one from "Sikkim" (collected by K. Bouk [Bauer et al. 1993]), one from "Darjeeling, Bengal, India", and one from "Rungeet Valley, British Sikkim" collected by Tom Barbour [Taylor 1960]).

Remarks: Pillai and Ravichandran (1999) identified eight additional specimens (including five larvae), of which seven are available at the Zoological Survey of India (ZSI), Kolkata and one at the Bombay Natural History Society (BNHS), Mumbai (Bombay), India. They did not examine the type specimens but relied on Taylor's (1960) description. Gower et al. (2017) re-examined the BNHS specimen and concluded that the specimen is not a congener of *I. sikkimensis*. Pending re-examination of type materials and ZSI materials, and a revision of the species, the species diagnosis below has been reproduced from the original description.

Species diagnosis: "A medium-sized species, characterized by 106-108 vertebrae; primary and secondary transverse folds 276-292; series of splenial teeth (9-9 or 10-10); tail very short, contained approximately 50 times in total length, bearing five or six folds from front of vent; tentacle near lip, closer to eye than to nostril. Scales sparse or absent in anterior half of body; two to four rows in each fold posteriorly" (Taylor, 1960).

Ichthyophis sikkimensis is one of the two unstriped forms of *Ichthyophis* described from India; it is a short-tailed species (distance behind vent, about 5 mm [Taylor 1960]). The other unstriped form from India is the long-tailed (distance behind vent, about 15 mm [Taylor 1960]) *Ichthyophis bombayensis* Taylor, 1960, distributed in the Western, and possibly the Eastern Ghats of the Indian peninsula (Ramaswami 1947, Gower et al. 2007).

Distribution: This species is known from Darjeeling in West Bengal and Sikkim in India, and Nepal (Taylor 1960, 1961, 1968; Pillai & Ravichandran 1999, Anders et al. 2002; Fig. 1). GPS localities reported for the species by Pillai & Ravichandran (1999) are not included in this work.

CHIKILIDAE

(i) Diversity: The family Chikilidae Kamei, San Mauro, Gower, Van Bocxlaer, Sherratt, Thomas, Babu, Bossuyt, Wilkinson, and Biju, 2012 is the most recent new family to be discovered in caecilian taxonomy and systematics. The discovery and description of a novel family was the direct result of focused and extensive soil-digging expeditions between 2006 and 2010 (Kamei et al. 2012) across NEI. The family is monogeneric, and as far as is known, Chikilidae is endemic to NEI (Kamei et al. 2012). Current taxonomy recognises four nominal *Chikila* species in the NEI states of Arunachal Pradesh, Assam, Meghalaya, Nagaland and Tripura (Kamei et al. 2012; Fig. 4)—*Chikila alcocki*, *C. darlong* and *C. gaiduwani* described by Kamei et al. (2012), and *Chikila fulleri* (Alcock, 1904).

(ii) Systematics and biogeography: The discovery of Chikilidae improved our knowledge of phylogenetic relationships within caecilians, and also resolved a biogeographical enigma in caecilian systematics that had persisted for over a century. *Herpele fulleri*, then placed in the catchall caecilian family Caeciliidae Rafinesque, 1814, was the first caecilian species described from NEI. The species was described by Alfred W. Alcock, a British naturalist who served as a Superintendent of the Indian Museum in Kolkata, West Bengal India in the 1890s, on the basis of a single specimen. The species was reallocated to the genus *Gegeneophis* Peters, 1880 (Indotyphlidae Lescure, Renous, and Gasc, 1986), a lineage now known to be exclusive to the Western Ghats of peninsular India (Gower et al. 2011), by Taylor (1968).

For over a century since its original description, *Gegeneophis fulleri* was accepted to represent the easternmost limit of the distribution range of the family Caeciliidae. The phylogenetic affinities of the unique teresomatan (teresomatans are the advanced [Nussbaum 1991] or higher [e.g., San Mauro et al. 2004] caecilians that lack true tails [Wilkinson & Nussbaum 2006]) specimen from NEI remained unknown; the distribution of the "lineage" remained a biogeographical conundrum, as there was no new information after the

species' original description. As a result of expansive (238 localities surveyed between 2006-2010) soil-digging surveys across NEI, Kamei et al. (2012) reported finding hundreds of teresomatan caecilians.

Morphological and molecular data and divergence time dating estimates indicated that NEI teresomatans were a previously overlooked, ancient lineage (ancestral divergence estimated at > 125 million years ago) with an interesting and unexpected sister-group relationship with the exclusively African herpelids, rather than to the Indian indotyphlids. The finding of an ancient endemic vertebrate lineage stimulated a novel perspective about NEI that was long-considered to be merely as a "gateway" (Mani 1995) between the Indo-Burma hotspots and Himalaya without its own distinctive and/or old endemic lineages. The discovery of Chikilidae however contested the gateway perception and showed that the region with its biotic assemblage might have a much more important role to play in understanding the global biogeography and phylogenetic affinities of caecilian fauna in particular, and biodiversity in general.

(iii) Biology: *Chikila* species in NEI have been found to be easier to locate during the heavy monsoons (beginning of June to end of July) when the animals likely migrate to near water sources for breeding (Kamei et al. 2013). Although not many surveys have been made outside the wet season, the few surveys made just before and after the arrival of the first few rains in May, and in January, far fewer animals were encountered in the dry seasons (pers. obs.). *Chikila* are oviparous, with direct development in the egg, i.e., no free swimming larval stage (Kamei et al. 2012).

A specimen of *Chikila gaiduwani* was reported with a clutch of five eggs. The colour of eggs (freshly laid eggs and later ontogenetic developmental stages) and the manner in which eggs are laid are similar to those described for *Ichthyophis*. With the exception of drier areas of nesting sites described for *Ichthyophis*, *Chikila* females with egg clutches are also found in similar microhabitats. *Chikila* species have been found with litters of hatchlings without yolk on the belly in the field (pers. obs.). Litter attendance could indicate some form of extended parental care as has been reported

for African species (*Boulengerula taitanus*, Kupfer et al. 2006; *Geotrypetes seraphini*, O'Reilly et al. 1998). Extended parental attendance might indicate that hatchlings are altricial and are dependent on mothers for nutrition (Kupfer et al. 2006) until they can feed independently. These speculations deserve more investigation. Nothing else is known about the biology of the *Chikila* spp. of NEI. Although the surveys (Kamei et al. 2012, 2013) have been biased towards unprotected areas, they have been mostly found in disturbed habitats such as around wet paddy fields.

(iv) Taxonomy: The following are brief taxonomic accounts of species and their distribution (Figs. 2, 5). *Chikila* species are not easily identified. For example, the total number of primary annuli (PAs), how far anterior secondary AGs (SAGs) appear, and the number of PAs subdivided by SAGs are generally useful taxonomic characters used for species diagnosis in teresomatan caecilians, but these characters overlap in the four *Chikila* species. Kamei et al. (2013) distinguished the four nominal species on the basis of external (measurements and meristics) and internal morphology in concordance with mitochondrial DNA data.

Abbreviations used: AG = annular groove, PA = primary annulus, PM = premaxillary-maxillary tooth, VP = vomeropalatine tooth (Kamei et al. 2013, Kotharambath et al. 2012a, Wilkinson & Kok 2010).

(a) *Chikila alcocki* Kamei, Gower, Wilkinson, and Biju, 2013 (Fig. 5A):

This species was described based on a series of 15 specimens--holotype (an adult female), from "Dhyutere (25.91528 N, 94.10528 E; 782 m asl), New Sendenyu village, Tseminyu subdivision, Kohima district, Nagaland, India.", seven paratypes (four adult females, three adult males) and seven referred specimens (three adult females, four adult males) collected from within the vicinity of the type locality (Kamei et al. 2013).

Species diagnosis: The adult colour pattern in this species is weakly bicoloured to almost unicoloured. Adult females are known to grow up to a total length of 271 mm, and males up to 255 mm. The AGs are weakly marked externally (despite strong myoseptal pigment under skin). Head is somewhat broad in dorsal view; eyes, both

in life and in preservation, are not visible. The shortest distance between choanae relative to the width of each choana at that point is narrow, with a $> 2.25 \times$ gap. The number of VPs and PMs are about the same, sometimes more VPs; the shape of the anterior end of the VP series in palatal view varies from an indented arc to being weakly angulate. The cloacal disc is unpigmented, and a pale patch extends anteriorly from the disc (Kamei et al. 2013).

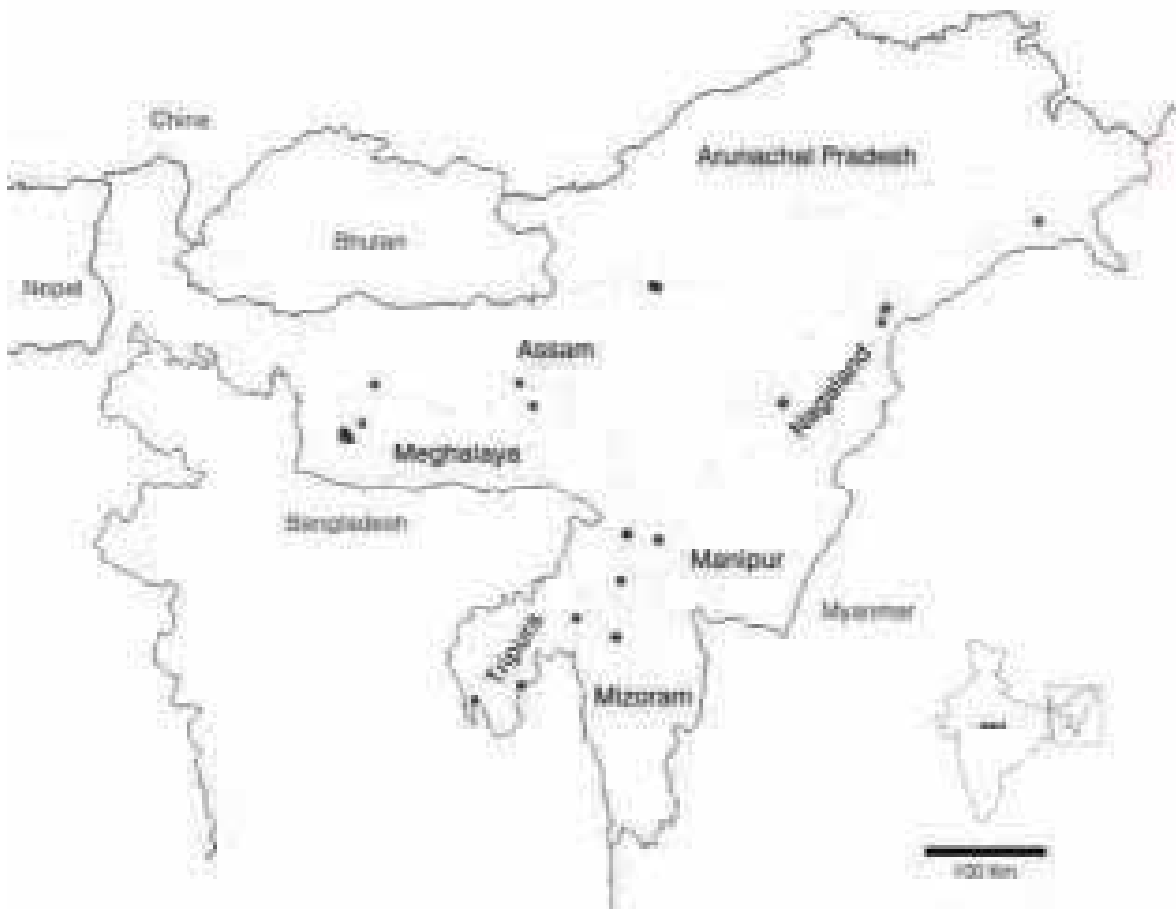
Distribution: This species is currently known from the type locality Dhyutere, New Sendenyu, Kohima district, Nagaland and its vicinities--Mon district in Nagaland, and Changlang district of Arunachal Pradesh (Kamei et al. 2013). See Kamei et al. (2013) for GPS coordinates of localities. The species is known from an elevational range of 320-900 meters asl, and is reported to be fairly abundant at the type locality (Kamei et al. 2012, 2013; Fig. 4).

(b) *Chikila darlong* Kamei, Gower, Wilkinson, and Biju, 2013 (Fig. 5B):

This species was described based on a series of 16 specimens--holotype (an adult female), from "Darlong (26.93722 N, 92.99611 E; 121 m asl), Seijosa, East Kameng district, Arunachal Pradesh, India.", ten paratypes (four adult females, six adult males) and five referred specimens (three adult females, two adult males) collected from the vicinity of the type locality (Kamei et al. 2013).

Species diagnosis: The adult colour pattern in this species is moderately bicoloured (but less so than *C. fulleri*). Among *Chikila* species, *C. darlong* attains the smallest adult size; the adults are not known to grow more than 200 mm in total length; adult females are known to grow up to a total length of 164 mm, and males up to 198 mm. The AGs are moderately marked. Head is acuminate in dorsal view; eyes, both in life and in preservation are generally distinct. The shortest distance between choanae relative to the width of each choana at that point is small, with a 1.25 to $2 \times$ gap. The numbers of VPs are fewer than PMs; the shape of the anterior end of the VP series in palatal view varies from a gentle arc to fairly angulate. Cloacal disc is unpigmented, with a pale patch extending anteriorly from the disc (Kamei et al. 2013).

Distribution: This species is currently



known from the type locality in Darlong, and from Pakke Tiger Reserve (approximately 3-5 km from the type locality), East Kameng district, Arunachal Pradesh, India (Kamei et al. 2012, 2013; Fig. 4). See Kamei et al. (2013) for GPS coordinates of localities. The elevational range of this species is 120-350 m asl. *Chikila darlong* is the first, and only caecilian species endemic to Arunachal Pradesh, and is the only known chikilid species found north of the Brahmaputra River.

(c) *Chikila fulleri* Kamei, Gower, Wilkinson, and Biju, 2013 (Fig. 5C):

This species was described by Alcock (1904) on the basis of a unique holotype specimen (a gravid female [Kamei et al. 2013]) that was "...discovered in a miscellaneous collection of snakes from Kuttal, 6 miles south-west of Silchar in Cachar", in Assam, India, purchased from Mr. C. B. Antram (Alcock 1904).

Remarks: Ordinarily, Alcock might have

been expected to assign his specimen to the Asian teresomatan *Gegeneophis* but he assigned it to an African genus *Herpele*; Alcock was likely misled by Boulenger's (1882) erroneous report that *Gegeneophis* lacked annular scales, a taxonomic error that Taylor (1961) corrected. Taylor (1968) was unable to examine the type specimen. For over a century, *C. fulleri* remained known only from the original description and the badly dehydrated and broken holotype (Kamei et al. 2013). Ahmad (2001) reported that the species was possibly extinct at the type locality. Kamei et al. (2012, 2013) reported 12 referred specimens (eight adult females and four adult males) from the type locality, and gave a revised diagnosis for the species based on the holotype and the new specimens.

Species diagnosis: The adult colour pattern in this species is moderately bicoloured (but more so than *C. darlong*). Adult females are known to grow up to a total length of 230 mm, and males up to 190 mm. The AGs are very strongly marked by distinct myoseptal

Figure 4: Map of North East India (NEI) showing the distribution of *Chikila* species in NEI. Localities are from (Kamei et al. 2013). *Chikila alcocki* = green spots, *C. darlong* = red, *C. gaiduwani* = pink, *C. fulleri* = dark blue.

pigment. Head is somewhat pointed in dorsal view; eyes, both in life and in preservation, are faint or not visible (except in the severely dehydrated holotype where they are clearly visible (Kamei et al. 2013). The shortest distance between choanae relative to the width of each choana at that point is small, with a 1.25 to 2 x gap. The number of VPs and PMs are about the same, sometimes more VPs; the shape of the anterior end of the VP series in palatal view is angulate. *Chikila fulleri* has some distinctive pigmentation patterns not seen in the other three congeners--presence of pale blotches on the chin, throat, and anterior most one to three PAs; the base of denticulations surrounding the vent are mostly pigmented; an unpigmented patch

anterior to the cloacal disc (as seen in the three congeners) is absent.

Distribution: This species is currently known from the type locality Kathal Tea Estate, Silchar, Cachar district, Assam, India as refined by Kamei et al. (2013), and in the vicinity (Chinglaeu, Bhubon Hills, Silchar) of the type locality; Joychandpur tower, Trishna Wildlife Sanctuary and Rambhadra, Gumti in South Tripura district; Vanghmun, Jampui Hills in North Tripura district (Fig. 4). The elevational range of this species is 10-600 m asl. See Kamei et al. (2013) for GPS coordinates of localities.

Chikila fulleri-like animals have been encountered in Mizoram (Buchangphai in Kolasib district and Lunglei in Mamit

Figure 5: *Chikila* species in life. A. *Chikila alcocki* female with an egg-clutch in captivity, B. *C. darlong*, afemale with hatchlings in captivity, C. *C. fulleri*, D. *C. gaiduwani*. Image 5B © Systematics Lab.



district) (Kamei et al. 2013) but the species-level identity of the specimens could not be confirmed, as the authors could not obtain the necessary permits for collecting voucher specimens (Kamei et al. 2013). However, the distribution map (Fig. 4) has included Mizoram.

(d) *Chikila gaiduwani* Kamei, Gower, Wilkinson, and Biju, 2013 (Fig. 5D):

This species was described based on a series of 10 specimens--holotype (an adult female), from "Diringa bisik, Silchol, Tebronggre village" (Kamei & Biju 2016) (25.61750 N, 90.23639 E; 519 m asl), Tura, West Garo Hills district, Meghalaya, India, five paratypes (four adult females, one adult male), and four referred specimens (all adult females) collected from the vicinity of the type locality.

Remarks: The original type locality "Teobronggre (Theobongiri) Coffee Garden" (Kamei et al. 2013) was subsequently corrected to Diringa bisik, Silchol, Tebronggre village (see Kamei & Biju [2016] for discussion). *Chikila gaiduwani* was named in honour of the author's father Mr Gaiduwan Gaipuziei Kammei (Kohima, Nagaland), in appreciation of him and Kamei's family who have supported immensely the author and team's research in northeast India (Kamei et al. 2013).

Species diagnosis: The adults of this species are strongly bicoloured. Adult females are known to grow up to a total length of 253 mm, and males up to 272 mm. The AGs are moderately marked. Head is broad in dorsal view; eyes, both in life and in preservation are not visible. The shortest distance between choanae relative to the width of each choana at that point is large, with a $<1.25 \times$ gap. The number of VPs and PMs are about the same, sometimes more VPs; the shape of the anterior end of VP series in palatal view forms a gentle arc (not angulate). Cloacal disc is unpigmented, and a pale patch extends anteriorly from the disc.

Distribution: This species is currently known from the type locality Teobronggre Coffee Garden, Teobronggre, Tura and surrounding areas in West Garo Hills district, and from Nongpoh, in Ri Bhoi district of Meghalaya; Dwarka, Goalpara district and Kanchiguli, Garbhanga, Kamrup district of Assam (Fig. 4). See Kamei et al. (2013) for GPS coordinates of localities. The

known altitudinal range is 86-538 meters asl.

Conservation

There is an acute lack of basic knowledge of NEI caecilians--a large majority of the general public is not aware of their existence, and this does not exclude the wildlife custodians of the region. The tiny section of the general public aware of caecilians' existence is locals in remote villages, largely the relatively uneducated farming community, living close to habitats (in and around agricultural fields, or forests) where caecilians are found. Unfortunately, a widespread misconception passed down through generations by oral traditions that caecilians are fatally venomous (Kamei, 2015 [unpublished]) have misled locals to have deep-rooted fears for caecilians, resulting infrequent intentional killing (Kamei et al. 2013, Kamei, 2015 [unpublished]; Fig. 6). Animals (including eggs and hatchlings) killed in agricultural fields are frequently encountered (Kamei et al. 2013, pers. obs.; Fig. 6). Gravid females, males with enlarged testes lobes, egg clutches and / or hatchlings, of both *Ichthyophis* spp. and *Chikila* spp., have almost exclusively been reported and/or collected during the monsoon (late May to mid August) season, (Kamei et al. 2009, 2012, 2013; Mathew & Sen 2009) leading to the premise that breeding period coincides with the peak agricultural activity, especially paddy farming (Kamei et al. 20013). Due to several reasons, including an acute lack of workers in this herpetological group in the region, caecilian field research requiring long hours of skilled and dedicated soil-digging surveys, and a resource shortage, there is no published quantitative data available to infer whether persecution by uninformed locals poses a threat to the conservation of NEI caecilian species. Sharing scientific knowledge and spreading awareness to dispel the myth that caecilians are venomous to the grossly un-/misinformed general public is imperative to create a practical and sustainable conservation impact, especially in NEI where the concept of conservation exists only as a far-fetched notion, or the realm reserved almost exclusively for large charismatic mammals. Kamei (2015 [unpublished]) conducted public outreach (Fig. 7) in NEI reaching out to a motley (relatively non-literate farmers and locals,



Figure 6: Examples of intentional killing of caecilians by local people. Image 6B © = Stephen Mahony.

school and college students, researchers, forest department staff) audience of over 6000 people, educating the general public about caecilians. However, NEI is a massive geographical region beset with numerous challenges, such as, generally very poor civil infrastructure, rough terrains, ethno-civil strife, insurgency and militancy, language barriers due to the huge diversity of ethnic groups, and others. Public outreach programmes will need to be extended and supported by the state forest custodians designated to safeguard the forests and wildlife of the country, if they are to bring about a reasonably widespread awareness among the general public to contribute to a significant and lasting conservation impact. Also, given that all NEI caecilians are endemic to NEI, and are restricted to small geographical locations, the need to escalate public awareness campaigns is urgent in the face of relentless habitat destruction.

Knowledge of conservation requirements for NEI caecilian fauna are severely

inadequate--all caecilian species are currently either listed as Data Deficient (DD) in the IUCN Red List (IUCN 2017) or, are yet to be evaluated against the IUCN Red List criteria. Survey of new areas for the occurrence and abundance of species particularly for those reported from only a small area (e.g., *Chikila darlong*, *Ichthyophis sendenyu*, *I. khumhzi*), or that have not been reported for several decades after the original description (i.e., *I. sikkimensis*), evaluating the presence and abundance of caecilian species in protected areas, obtaining more ecological data particularly reproduction and habitat requirements, are necessary to determine more reliable and accurate conservation assessments. Although the occurrence of species in multiple localities (e.g., Kamei et al. 2013), and their persistence in disturbed habitats (e.g., Kamei et al. 2009, 2013) may provide some consolation that they might not be immediately threatened, this idea relies primarily on the assumption of a reasonable range size of the species.



Figure 7: Figure 7. Public outreach to different sections of society. A. Hands-on demonstration to locals that caecilians are harmless, and that they do not bite. A girl is being encouraged to hold a live caecilian in her hands to dispel her fear for caecilians. B. Sharing knowledge with locals about caecilians through pictures and informal conversation. C. The author with a batch of new recruits of forest guards in Tripura Forest Academy after field demonstration on how to search for caecilians and what kinds of habitats. D. The author with school children aged between six and fourteen after a popular talk on caecilians. Image 7C copyright = Pallab Chakraborty, 7D = Adventure Club, Tamenglong (ACT), Manipur, India.

Challenges

The foremost challenge to caecilian field-based research is finding specimens since NEI caecilians are generally secretive, living in concealed habitats that can only be found by digging the soil, raking through organic litter, or flipping rotting logs and rocks. On rare occasions one might have the good fortune to sight an *Ichthyophis* individual above soil surface during or after heavy downpours, but *Chikila* spp. are dedicated burrowers, and to the best of my knowledge, they have not been encountered above soil surface during the caecilian-specific surveys (Kamei et al. 2012, 2013; pers. obs.). Caecilian field work can be very labour-intensive because individuals of some species in some places can be sparsely encountered, and one may not find any individuals despite extended and intensive searching (several hours per day for several days) in habitat that appears suitable. This rarity of animal sightings and the difficulties involved in finding them are

contributing factors to under representation of specimens in natural history collections and in turn account for several caecilian species known from only small sample sizes (one to a few specimens, e.g., Nussbaum & Wilkinson [1989], Wilkinson et al. [2007]).

Caecilian taxonomy has numerous limitations on several fronts. One of the fundamental difficulties is that morphologically, caecilians can be cryptic (Gower & Wilkinson 2005, Nussbaum & Wilkinson 1989, Wilkinson et al. 2007). The dearth of morphological characters due to their limbless and tubular body plan compounds this taxonomic problem—it is difficult to tell most species apart by mere “eyeballing” for subtle differences in their external morphological characters. Integrative taxonomy (Dayrat 2005, Padial et al. 2010, Wheeler 2005) is indeed the way to go but is confronted with several difficulties, e.g., scarcity of specimens for soft and / or hard (destructive) anatomical work; lack of DNA samples for historically described species; difficulty of obtaining

paratopotypes, or, from near by the type localities because the recorded provenance of several of the historical species are imprecise, e.g., the type locality of *Ichthyophis sikkimensis* is just given as "Darjeeling, [West Bengal,] India", and it is unclear whether this referred to the town or the district of Darjeeling; there is not enough native experts to comprehensively work on this poorly studied vertebrate group, especially for India that has approximately 40 known native caecilian species. Type specimens are scattered in international (mostly European) natural history collections. The cost to go to examine type specimens in overseas collections is prohibitive deterring many from attempting a modern revision. International museum collections are hesitant to provide specimens (unless for destructive sampling) on loans to India, because import of biological specimens has been relatively straightforward but obtaining export permits for any biological material has been unreliable in the past. Some of the (type) specimens that are deposited in Indian natural history collections are in very poor condition (e.g., Kamei et al. 2013, Kamei & Biju 2016, Wilkinson et al 2007) reducing their value for meaningful and/or comparative taxonomic study. Although taxonomy is a fundamental basis for all biological science and its application (Sluys 2013), including conservation biology, today much of taxonomy is facing a crisis alack of prestige and resources that is crippling the continuing cataloguing of biodiversity (Godfray 2002). Securing funding for taxonomy has only become increasingly difficult.

Indian native scientists are also faced with sometimes disheartening processes of obtaining various kinds of necessary permits (e.g., Bagla 2006, Kamei et al. 2013, Madhusudan et al. 2006, Varshney 2015) that have been put in place to safeguard biodiversity. Excessive restrictions, red-tapism (e.g., Madhusudan et al. 2006, Varshney 2015), and lack of respect for scientific merit are major hindrances to biodiversity-related research, which cannot be done in isolation. Early-career scientists are also faced with the subtle form of discrimination that they are not "renowned" enough to be issued a permit despite publications in prestigious international peer-reviewed scientific journals. Often, a scientist is also faced with the conundrum



"which comes first, chicken or the egg?"

—permit issuing authorities would require a signed contract for funding, while funding agencies will require permits to have been already obtained prior to funding. Basic research such as taxonomy is still considered largely irrelevant to conservation (Madhusudan et al. 2006, pers. obs.) and this knowledge deficiency seriously hinders taxonomists, which in consequence, compromises the documentation of biodiversity. A forest department initially denied issuance of permit to the author on the grounds that "taxonomy and systematics research" do not contribute to conservation, while another state forest department clearly denied collection permit for a few sample vouchers insisting that community ecology be done in situ without collection. Bureaucratic obstacles to purely academic scientific research manifested in numerous manners and forms not only work to the detriment of India's progress of understanding the diversity but also act as shackles in the strive towards fulfilling the Fundamental Duty under the Constitution of India article 51A. (j), "To strive towards excellence in all spheres of individual and collective activity, so that the nation constantly rises to higher levels of endeavour and achievement" (The Constitution of India, 1950). I hope that future academic research will be facilitated more constructively.

Political volatility, guerilla warfare, and social unrest in several parts of NEI also greatly impede academic scientific work. The author's attempts to conduct public outreach programme as part of Community Education Project (Kamei, 2015[unpublished]) in schools in Imphal, Manipur, and in Tura, Meghalaya for two simultaneous (2014, 2015) years were

Feeding habits of Caecilians are poorly known. *Ichthyophis* sp. feeding on earthworm
Photo credit: Vivek Sarkar

prevented due to the sudden eruption of violence and curfews. Several regions in NEI still remain to be explored because of erratic and / or orchestrated violence that erupts all too often, or because many of the pockets of original habitat in NEI are also strongholds of the countless militias in operation. Volatilities further skyrockets the cost of logistics, if at all available. Such unsafe scenarios also put women field biologists in greater risk for personal safety.

Conclusions

Knowledge of NEI caecilian true diversity still remains incomplete. Caecilians new to science could well be found in the remaining vast expanse of NEI that has not been covered by Kamei et al.'s (2009, 2012, 2013) and Mathew & Sen's (2009) surveys. What is known of the 12 recognised species from NEI is rather pitiable—for all of the named species, we do not know the real distribution range, presence and abundance in protected areas, breeding biology, habitat requirements, diet, how well adapted they are to human-disturbed areas, ecological requirement, captive management (whether they can be captive bred can be important for future conservation actions), genetic structure of their populations, and so forth. We do not know the phylogenetic relationships of the NEI *Ichthyophis* assemblage or, whether their DNA contains information that might help resolve more precisely an important question in Southeast Asian caecilians' biogeography (Duellman & Trueb 1986, Feller & Hedges 1998, Gower et al. 2002, Hedges et al. 1993, Nishikawa et al. 2012, Wilkinson et al. 2002,), or shed light on the roles played by biological barriers in the determining the present (and past) distributions.

Gower et al.'s (2017) recent conclusion that *I. sikkimensis* is restricted to only the Indian states of Sikkim and West Bengal (Taylor 1960) and Nepal (Anders et al. 2002), and is not present in the Western Ghats as reported by Pillai & Ravichandran (1999) underscores the significance of taxonomy in defining distribution patterns, resolving biogeographic anomalies, and, consequently, for assessing the conservation status of a species (see also Gower et al. 2015, Kotharambath et al. 2012b). The taxonomic validity of a few of the striped forms of *Ichthyophis* from NEI is questionable; a modern systematic review of the group is required.

Datta et al.'s (2008) "empty forests" scenario in Namdapha National Park, Arunachal Pradesh, India would not be unusual in other parts of NEI. Studies on forest depletion and habitat destruction due to different reasons are still largely unavailable for a large majority of NEI forests, but the few published studies (e.g., Kushwaha et al. 2011, Lele & Joshi 2008, Reddy et al. 2013) all indicate a forbidding scenario. The agricultural practise of slash and burn (e.g., Lele & Joshi 2008, Singh, & Borthakur 2015, Yadav et al. 2012), driven largely by the exponential human population growth, presents one of the most formidable challenges to wildlife conservation in NEI. Ethno-civil strife (e.g., Velho et al. 2014), insurgency (e.g., Reddy et al. 2013), increasing expanse of exotic vegetation (e.g., Puyravaud et al. 2010), and corruption (e.g., Laurance 2004) are other important factors that continue to threaten NEI's biodiversity unabated. Needless to say, biodiversity research is the need of the hour if our posterity is to get the privilege of relishing the diminishing nature's bounty. Increase in the scale of research in lesser-known vertebrate groups such as caecilians is clearly warranted. I hope that members of *Homo sapiens* will become more sympathetic to caecilians (and all wildlife), that the younger generation become more inspired to consider biodiversity and conservation science as viable career options, and that the bureaucracy become more compassionate towards the academic research community, to respect scientific merit, to foster global scientific collaboration, and to appreciate the benefits of international cooperation.

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Status and Distribution of Amphibians in the Andaman & Nicobar Island Archipelago

Abstract

The Andaman & Nicobar Island Archipelago in the Bay of Bengal harbour a unique herpetofauna. We surveyed 23 islands across this archipelago, using a combination of methods, to estimate species richness, abundance, and distribution of amphibians. We recorded 17 species of frogs from these islands. Despite low species richness, frog densities were generally high but with high variability. Seven species were found to be endemic to the Archipelago, with five of them endemic to the Andaman Islands. Using the data on abundance, distribution, and natural history observations, we examine the conservation status of amphibians in these islands and the potential threats faced by them. We also illustrate some of the taxonomic issues that can hamper ecological studies and conservation efforts, and stress the need for taxonomic revisions for many taxa.

Introduction

Islands have had a major role in developing our understanding of the origin of biodiversity. Small islands have low species richness of most taxa due to the combined effects of isolation from source habitats, small geographic area, relatively young age of islands, and stochastic disturbance events on habitats. These factors create relatively simpler ecosystems (compared to continents) where ecological and evolutionary phenomena are often stark. However, most islands are not considered as hotspots of amphibian biodiversity. Majority of amphibian species are dependant on fresh water at some stage of their lives, a resource that is scarce in most small islands. Immigration of organisms to islands happen through active (across land bridges, flying, or swimming) or passive (rafting on flotsam, floating islands, or wind) dispersal. Almost all amphibian species have hygroscopic skin and are highly intolerant to salinity. This makes dispersal across ocean barriers a highly unlikely event for most amphibian species. More than anything else, this could be the single most

important factor restricting the diversity of amphibians in islands. However, several recent studies have provided evidence for multiple oceanic dispersals by amphibians (Bell et al. 2015; de Queiroz 2005; Vences et al. 2003). Thus, many isolated islands harbour small but unique amphibian fauna. As the phylogenetic species concept finds widespread acceptance, it is likely that many isolated island populations of species that are currently considered as widespread species will be recognized as evolutionarily distinct lineages. Identification of such island lineages as distinct species will result in a marked increase in the number of

Higher elevation in saddle peak supports stunted wet evergreen forest.

S. Harikrishnan¹, Karthikeyan Vasudevan² & S. K. Dutta³

¹Saurashtra University, Rajkot.

²Laboratory for the Conservation of Endangered Species (LaCONES), Centre for Cellular and Molecular Biology, Hyderabad.

³Centre for Ecological Sciences, Indian Institute of Science, Bengaluru.

Corresponding author e-mail: s.harikrishnan09@gmail.com



endemic island amphibian species. This is true in the case of the largest island archipelago system in India, the Andaman & Nicobar Islands.

The Andaman & Nicobar Island Archipelago

The Andaman & Nicobar Islands consist of 556 islands, islets, and rocks, covering 8249 km², situated in the eastern part of the Bay of Bengal (Anonymous 2007). These islands are part of a chain of mountains sprawling in a great arc between Cape Negrais in Myanmar and Achin Head in Sumatra about 155 km southeast of Great Nicobar Island. The length of the island chain itself is about 1126 km (Biswas & Sanyal 1980; Das 1999; Smith 1940). It is a part of the Great Alpine-Himalayan System (Karunakaran et al. 1968). The northward movement of the Indian tectonic plate, opening the Andaman Sea with its outer arc overriding the Indian plate, lead to the emergence of these islands (Lee & Lawver 1995). Thus, they are 'plate-boundary islands' and are highly tectonically active (Whittaker & Fernández-Palacios 2007). Cenozoic plate reconstructions of Southeast Asia indicate that the emergence of these islands above sea level happened only during the late Miocene (10 million years before present) and the present configuration was achieved about 5 - 10 million years ago (Lee & Lawver 1995). The Mentawai Islands off the coast of Sumatra appear to be a continuation of the Nicobar Islands (Weeks et al. 1967; Rodolfo 1969). While the Nicobar Islands appear to be truly oceanic in nature, surrounded by deep channels, the Andaman Islands at their northern tip might have been connected to mainland Asia during the Pleistocene (Ripley & Beehler 1989). The mean annual rainfall in these islands exceeds 3000 mm. The highest amount of rainfall is in southernmost islands with no distinct dry season, whereas the northern islands show greater seasonal variation (Biswas & Sanyal 1980, Das 1999).

Owing to this high precipitation and their tropical location, the predominant vegetation type in these islands is wet evergreen forest. There are 11 major forest types in these islands (Champion & Seth 1968). These are: 1) Giant evergreen forest 2) Andaman tropical evergreen forest 3)



Southern hilltop tropical evergreen forest 4) Cane brakes 5) Wet bamboo brakes 6) Andamans semi evergreen forest 7) Andamans moist deciduous forest 8) Andamans secondary moist deciduous forest 9) Littoral forest 10) Tidal swamp forest 11) Submontane hill valley swamp forest (Champion & Seth 1968). These islands form parts of two global biodiversity hotspots: the Andaman Islands are a part of the Indo-Burma hotspot and the Nicobar Islands are part of the Sundaland hotspot (Myers et al. 2000).

Based on their origin and connectivity in the past, these islands can be divided into two major groups: the Andaman Islands in the northern part and the Nicobar Islands in the southern part. Ten Degree Channel, named so for the latitude at which it is located, separates these island groups. This channel is about 1000 m deep and approximately 140 km wide. This has ensured that the Andaman Islands and the Nicobar Islands remained separated from each other even during maximum sea level drops during the Pleistocene (Lee & Lawver 1995). The deep Andaman Sea on the east separates this island chain from Southeast Asia and The Bay of Bengal separates it from the Indian subcontinent.

Towards the north, Great Coco Island which is the northern-most island in the Andamans (geologically) is separated from Preparis Island which is located on the outer continental margin of Southeast Asia, by a channel about 76 km wide and approximately 500 m deep. The distance between Cape Negrais of Myanmar and Great Coco Island is about 215 km, although

Red-eared frog *Hylarana erythraea* from Great Nicobar island – this species is not been recorded post-tsunami survey in Nancowry group.

the sea is less than 100 m deep along most of this separation. The northernmost island in the Andamans administered by India is Landfall Island, which is about 40 km southwest of Little Coco Island. The highest peak in the Andaman and Nicobar Archipelago is Saddle Peak, which reaches 732 m above mean sea level (hereafter asl), and is in North Andaman Island.

Narcondam and Barren are the easternmost islands in the Andaman Islands, though they are volcanic in origin and have never had land connection with other islands in the Andamans. North Sentinel Island is the westernmost island in the Andamans.

Within Andamans, there are further divisions such as North Andaman, Middle Andaman, Baratang, and South Andaman, which, together with numerous adjoining islands are called Great Andamans. A major hill range occurs on the northeast part of south Andaman, comprising of several peaks such as Mt. Harriet (365 m asl), Mt. Carpenter (373 m asl), Mt. Goodridge (377 m asl), Mt. Koyob (460 m asl), Mt. Hext (424 m asl) and Mt. Warden (422 m asl) (Das 1997; Das 1999). Ritchie's Archipelago is a cluster of islands situated towards the east of Great Andamans. The southernmost island in the Andamans is Little Andaman Island, about 140 km north of Car Nicobar Island. Little Andaman is approximately 55 km south of South Andamans. Everywhere within the Andaman Islands, with the exception of Barren and Narcondam, the sea is relatively shallow (50-100 m), which means that all these islands were interconnected during major sea level changes in the Pleistocene.

The Nicobar Islands consist of 23 islands south of the Ten Degree Channel. The total land area of these islands is 1841 km². Three clusters of islands can be identified in this group. The northern cluster consists of only two islands, Car Nicobar and Batti Malv. The central cluster is collectively known as Nancowry group consisting of ten islands. Sombrero Channel separates the Nancowry group from the southern cluster consisting of Great Nicobar, Little Nicobar, and nine much smaller islands. Great Nicobar is the southernmost island in this group and is only about 300 km northwest of Sumatra. The Great Channel between Great Nicobar and Sumatra is more than 1000 m deep, which rules out any possibility of land

connections in the recent past.

Car Nicobar is a relatively flat island with a maximum elevation of 30 m. It is also the most densely populated island and coconut plantations and orchards dominate the landscape. Patches of evergreen forests occur intermittently throughout the island. Nancowry group is hillier; Maharani Peak in Tillangchong Island reaches about 300 m asl. Islands in the Nancowry group also have evergreen forests, though in many areas, they have been replaced by secondary forest. They also support extensive grasslands on hilltops. Islands in the southern group have lower human population density and are extensively covered in wet evergreen forests. Mt. Thullier in Great Nicobar is the highest peak in the Nicobar Islands, reaching about 642 m asl.

Amphibians of the Andaman & Nicobar Islands

Herpetological exploration in the Andaman & Nicobar Islands started in the middle of the 19th century when the Austrian 'Novara' expedition collected natural history specimens from several islands in this archipelago (Steindachner 1867). The first amphibian species recorded from these islands was the toad *Docidophryne spinipes* Fitzinger 1861"1860" from the Nicobar Islands, currently considered as a synonym of *Duttaphrynus melanostictus* (Schneider, 1799). This was followed by the discovery of *Hylorana nicobariensis* Stoliczka, 1870 (current name *Amnirana nicobariensis* (Stoliczka 1870)). In the same paper, Stoliczka also described *Rana gracilis* var.

Indian bull frog *Hoplobatrachus tigerinus*, an invasive species that has established itself in north, middle and south Andaman.



andamanensis Stoliczka, 1870 and *Rana gracilis* var. *nicobariensis* Stoliczka, 1870, which are both currently considered as valid species under the genus *Fejervarya* Bolkey, 1915 (*Fejervarya andamanensis* & *Fejervarya nicobariensis*). Following these early discoveries, the amphibian fauna of these islands remained largely unstudied. Smith (1940) reported the presence of only four species of frogs from these islands, including *Rana doriae* (= *Limnonectes doriae*). Post 1947, there were fresh attempts at collection and exploration of biodiversity in these islands. The first Microhylid frog described from these islands, *Kaloula baleata ghoshi* Cherchi, 1954 was described from Little Andaman Island. This was followed by the description of a second species of Microhylid endemic to these islands, *Microhyla chakrapanii* Pillai, 1977 along with a single record of *Micryletta inornata* (Boulenger, 1890) (Pillai 1977). Whitaker (1978) reported the presence of nine species of frogs from these islands, including *Rana erythraea* Schlegel, 1837 (= *Hylarana erythraea*), *Rana tigerina* Daudin, 1802 (= *Hoplobatrachus tigerinus*), *Rana breviceps* Schneider, 1799 (= *Sphaerotheca breviceps*) and *Microhyla rubra* (Jerdon, 1854), though the last two species have not been recorded in the islands since and was probably based on specimens of other species. Though a new species of toad, *Bufo camortensis* Mansukhani & Sarkar, 1980 was described from Camorta in the Nicobar Islands, it is now considered a synonym of *Duttaphrynus melanostictus* (Schneider, 1799) (Crombie 1986; Mansukhani & Sarkar 1980). Das (1996a) added *Rana chalconota* (Schlegel, 1837) (= *Chalcorana chalconota*) to the amphibian fauna of Great Nicobar, while also mentioning that the population belonged to the subspecies *R. chalconota raniceps* (Peters, 1871). In the 1990s, three new species of frogs were discovered from the Andaman & Nicobar Islands. *Polypedates insularis* Das, 1995 and *Limnonectes shompenorum* Das, 1996 were described from Great Nicobar Island, while *Rana charlesdarwini* Das, 1998 (= *Ingerana charlesdarwini*) was described from Mt. Harriet National Park in South Andaman Island (Das 1995; Das 1996b; Das 1998). Das (1999) listed 19 species of anuran amphibians from these islands based on collections, and dispelled several

misidentifications. There were doubtful records of *Hoplobatrachus tigerinus*, a species thought to be introduced deliberately by humans from mainland India into the Andaman Islands as early as 1978 (Whitaker 1978). Recently, the occurrence and spread of this species was confirmed in the Andaman Islands (Harikrishnan & Vasudevan 2013). Most recently, an endemic, semi-arboreal Bufonid, *Blythophryne beryet* Chandramouli et al. 2016 was described from the Andaman Islands. This is the only amphibian genus endemic to the Andaman & Nicobar Islands (Chandramouli et al. 2016). These islands do not have any caecilian and salamander species.

Most studies of amphibians in these islands have been rapid surveys or anecdotal records of species. So far, there is little information on abundance, distribution, and status of these species. At least one species, *Ingerana charlesdarwini*, is listed as a Critically Endangered species by IUCN, based on its supposed restricted distribution in Mt. Harriet and Saddle Peak National Parks. In this study, we surveyed 23 islands in the Andaman & Nicobar Islands for presence and abundance of amphibian species.

Methods

Geographic distribution of amphibian and reptile species in Andaman & Nicobar Archipelago was assessed through both field surveys and secondary sources (museum specimens and literature). Field surveys were carried from October to June, avoiding peak monsoon. This is a weakness of studies in these islands due to extreme logistical difficulties in small islands during the monsoon season. To assess the presence of species in each island quadrat surveys (see below), visual encounter surveys (VES) and pitfall traps with drift fences were used. Each VES was one hour long, where we walked the forests at a slow pace looking for amphibians after sunset (between 6 p.m. and 9 p.m.). Pitfall traps with drift fences were only attempted in four islands due to logistic constraints. These were 25 cm × 30 cm buckets buried in the ground with the rim flush with the ground, and a 30 cm high plastic sheet erected vertically above them to act as a drift fence. A few holes, small

enough to prevent animals escaping but large enough for the passage of water, were put at the bottom of the buckets to drain rainwater. A 20 m long plastic sheet was used as drift fence with buckets placed at 5 m intervals. The bottom of the fence was buried in the soil to prevent animals slipping under it. These were checked twice a day and any animal found in the buckets was captured, identified, and released slightly away from the fence. Pitfall traps were not attempted in all islands as they were labour intensive and provided very few unique records. Any species found opportunistically was also recorded. We also

used the locality data of specimens in the collection of Zoological Survey of India, Kolkata and Zoological Survey of India, Port Blair. In addition, we used published literature to obtain data on distribution of species. From all such records, distribution of species across islands and species richness in each island was enumerated. Fifteen islands in the Andaman Islands were surveyed from March 2010 to December 2012. Eight islands in the Nicobar Islands were surveyed thus from March 2008 to March 2009, from December 2012 to May 2013, and from December 2013 to February 2014. For other islands, data from secondary sources were used.

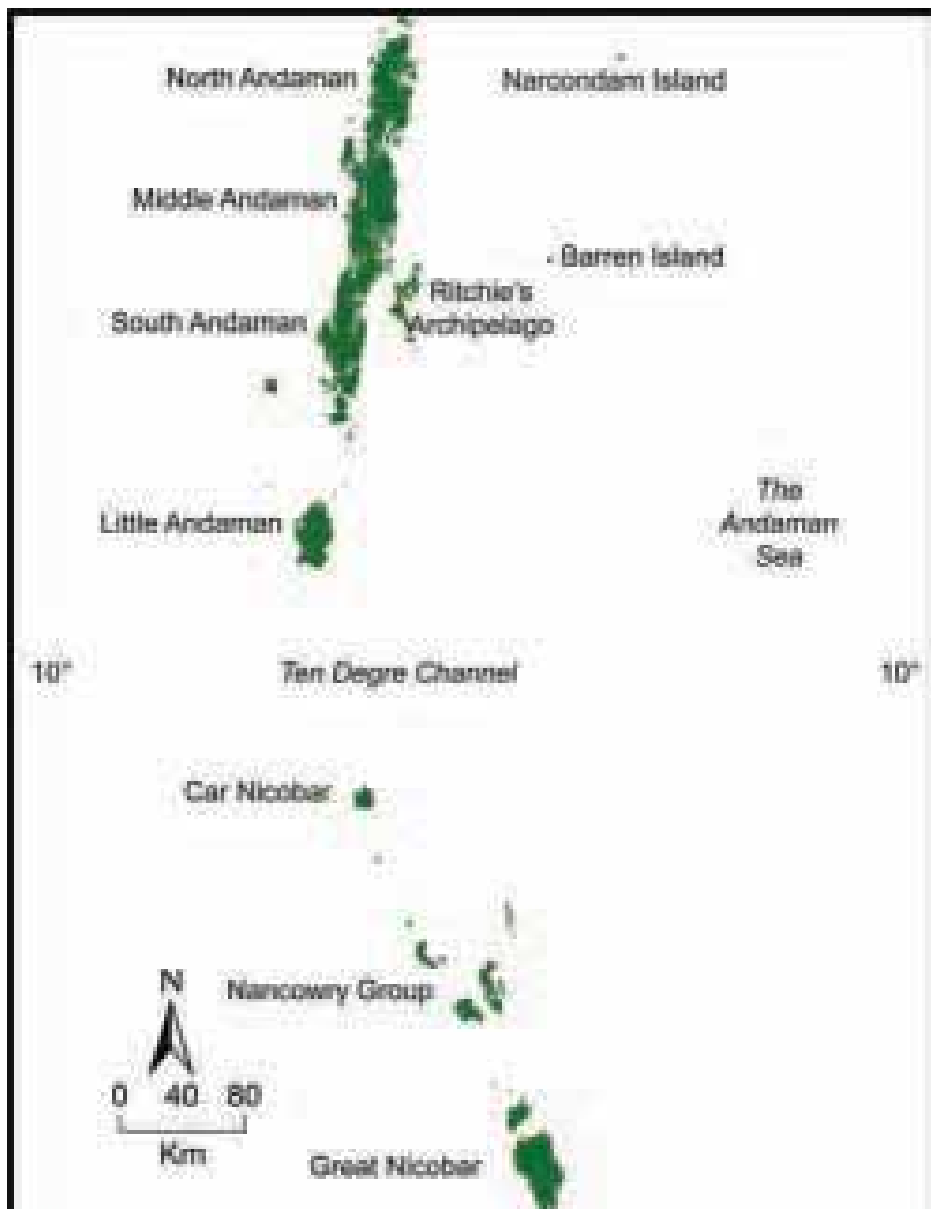


Figure 1: The Andaman & Nicobar Islands with major islands labelled. North of Ten Degree Channel, the islands are collectively called the Andaman Islands, and south of it are the Nicobar Islands.

To estimate abundance of amphibian species, we sampled bounded quadrats of dimension 10 m × 10 m (Harikrishnan & Vasudevan 2015). These were demarcated with a 50 cm high plastic sheet erected to prevent the escape of animals during sampling. The procedure was modified from Rodda et al. (2001) and explained in detail in Harikrishnan et al. (2015). All herbaceous vegetation and leaf litter on ground was removed and examined carefully for amphibians. Quadrats were only sampled in evergreen and semi-evergreen forests and therefore did not sample species that were found in other habitats (wetlands, marshes, ponds etc).

Results

We recorded 17 species of amphibians during our field surveys. Species were identified in the field using descriptions in published literature. Due to the lack of taxonomic clarity in many species descriptions, some species are tentatively

identified and some could only be identified to genus level. We recorded 9 species of frogs from the Andaman Islands and 10 species of frogs from the Nicobar Islands. Two species of frogs, *Duttaphrynus melanostictus* and *Fejervarya cancrivora*, occurred in both the island groups. Species richness increased with increasing island size in both the island groups ($\text{Log } S = 0.25\text{log}A + 0.13$, $R^2 = 0.59$, $F = 32.33$, $df = 22$, where S is species richness and A is island area). Many small islands did not have amphibians, and there was greater variation in species richness in small islands compared to large islands (Fig. 2). Small islands had amphibians only when they were close to larger islands (e.g. Alexandra). Species richness was also influenced by the presence of human habitations in large islands, through anthropogenic introduction of species. Species richness of individual islands is summarized in Table 1.

Table 1: Summary of species richness of amphibians in various islands in the Andaman & Nicobar Archipelago. (* Species occurrence data from past surveys). Abbreviations: BQ – Bounded Quadrats sampled, Y – Yes, N – No, na – Not Available (where secondary data was used).

	Island	Area (km ²)	Species richness	BQ	Surveyed elevation range	Perennial fresh water
Andaman Islands	North Andaman	1375.99	7	Y	0-700	Y
	South Andaman	1350.82	11	Y	0-350	Y
	Little Andaman	734.39	6	Y	0-131	Y
	Rutland	137.17	6	Y	0-400	Y
	Havelock	113.93	4	Y	0-66	Y
	Neil Island	18.9	4	Y	0-29	Y
	Long Island	17.9	7	Y	0-56	N
	Tarmugli	11.5	1	Y	0-33	N
	Alexandra	3.6	3	Y	0-32	Y
	Hobday	3.6	1	Y	0-48	N
	Redskin	3.3	1	Y	0-53	N
	Boat	2.8	0	Y	0-30	N
	Snob	0.22	0	Y	0-33	N
	Chester	0.09	0	Y	0-7	N
	Grub	0.03	0	N	0-15	N
Nicobar Islands	Great Nicobar	1044.54	9	Y	0-203	Y
	Camorta	188.03	4	Y	0-50	Y
	Katchal	174.3	4	Y	0-53	Y
	Little Nicobar*	159.02	6	N	na	Y
	Car Nicobar	126.91	4	N	0-20	Y
	Tarasa*	101.4	4	N	na	na
	Nancowry	66.82	3	N	0-20	Y
	Trinkat	36.26	3	N	0-10	N
	Tillangchong*	16.83	3	N	na	na
	Bompoka*	13.3	1	N	na	na
	Chowra*	8.28	0	N	na	na
	Kondul	4.66	1	N	0-10	N
	Menchal	1.5	1	N	0-5	N
	Pilo Milo*	1.29	4	N	na	N
	Pigeon	0.5	0	N	0-10	N

Nicobar Islands																
	CAR	CHO	BOM	TIL	TAR	KAT	TRI	CAM	NAN	PIL	LNI	MEN	KON	PIG	GNI	
<i>Duttaphrynus melanostictus</i>	+		+	+	+	+	+	+	+	+	+		+		+	
<i>Microhyla heymonsi</i>											+				+	
<i>Fejervarya cf. nicobariensis</i>	+				+	+	+	+	+							
<i>Fejervarya cancrivora</i>											+				+	
<i>Limnonectes shompenorum</i>										+	+	+			+	
<i>Limnonectes sp.</i>															+	
<i>Hylarana erythraea</i>	+			+	+	+	+	+							+	
<i>Amnirana nicobariensis</i>	+			+	+	+		+	+	+	+				+	
<i>Chalcorana cf. chalconota</i>															+	
<i>Polypedates insularis</i>										+	+				+	

- 1 – These species were reported by earlier authors based on specimens. They were not recorded in our surveys.
2 – Introduced species, which seems to have established in the Andaman Islands.

Forty-five bounded quadrats were sampled in the Andaman Islands and 16 in the Nicobar Islands, recording 302 and 34 individuals respectively. The bounded quadrats used in the analyses are only those from islands where amphibians occurred, as inferred from quadrats, or any other methods (VES, opportunistic records, historical records etc). Average density of frogs in evergreen forests was 720 ± 102

individuals/ha in the Andaman Islands, and 210 ± 190 individuals/ha in the Nicobar Islands. Frogs occurred at low abundance in many small islands, but this could be an artifact of the sampling period mostly covering post-monsoon and dry seasons. There is a large variation in abundance among quadrats, indicating the possibility of patchy or clustered distribution of individuals (Table 3). Therefore, the

Stream in a rain forest in Great Nicobar island



abundance estimates reported here for individual species may not reflect the true population abundance. The most abundant species in quadrats was *Limnonectes* cf. *doriae* (455 ± 913 individuals/ha). This species was abundantly recorded in leaf litter in evergreen and semi evergreen forests in the Andaman Islands. In the Nicobar Islands, the most abundant species was *Amnirana nicobariensis* (125 ± 161 individuals/ha) (Table 3). Several species of frogs were not recorded in quadrats, such as the arboreal *Polypedates insularis*, even though they were encountered commonly in other habitats.

Discussion

Species richness & distribution of amphibians

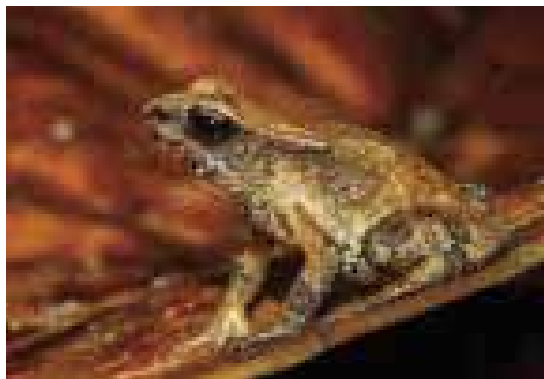
Our survey recorded 17 species of frogs across the Andaman & Nicobar Islands. This included the new species *Blythophryne beryet* (Chandramouli et al. 2016). The two species of Microhylids that we did not encounter are known only from single specimens. During post monsoon and summer months when the surveys were conducted, they occurred in low abundances. While quadrat surveys enabled the estimation of densities of rainforest species, other methods will have to be employed to estimate population sizes of species inhabiting other habitats. *Ingerana charlesdarwini*, a species considered as Critically Endangered (IUCN, 2014), was the second most abundant species of frog in evergreen forests in Andaman Islands where it occurred, with average density of 129 ± 277 individuals/ha. This species was also more widespread than previously recorded, occurring in at least ten islands. There are unsurveyed areas in Middle Andaman Island where this species could potentially occur. It should be noted that this is based on the assumption that individuals that were morphologically similar to the description of *Ingerana charlesdarwini* belonged to that species. Further taxonomic studies are necessary to determine whether all the islands harbour the same species or multiple cryptic species. In the Nicobar Islands, *Amnirana nicobariensis* was the most abundant

species in evergreen forests (125 ± 161 individuals/ha), while *Duttaphrynus melanostictus* was the most widespread species, occurring in almost all habitats. The high variability of all abundance estimates for frogs indicate a patchy distribution of amphibians in forests in the Andaman & Nicobar Islands. Therefore, improving the precision of abundance estimates might require a cluster sampling based approach.

Conservation status of amphibians in islands

There are seven species of frogs that are endemic to the Andaman & Nicobar Islands (Table 3) (Fig. 3). All these species, even those occurring in multiple islands, have distribution ranges of less than 5000 km². At least three of these, *Blythophryne beryet*, *Ingerana charlesdarwini*, and *Polypedates insularis* are primarily forest species, not recorded in human modified landscapes. *I. charlesdarwini* is the only species currently considered as Critically Endangered, though with its relatively high local abundance, it might be possible to lower the threat status classification of this species. Two species, *Fejervarya nicobariensis* and *Polypedates insularis*, are currently considered endangered based on their very small geographic distribution. The endemic *Microhyla chakrapanii* and the non-endemic *Limnonectes doriae* are data deficient species. Majority of other frog species occurring in the Andaman & Nicobar Islands are considered Least Concern (Table 3). *Hylarana erythraea*, a Least Concern species inhabiting ponds and wetlands, was recorded from southern group of islands in the Nicobars, and Car Nicobar Island, but not in the Nancowry group of islands. A survey conducted prior to the tsunami of December 2004 found these frogs in Nancowry group as well, but there have been no further records (Vijayakumar 2005; Vijayakumar & Choudhury 2006; Harikrishnan et al. 2011). We conducted surveys in the Nancowry group in October-December 2008, March-April 2013, and again in January 2014, but failed to record this species. Inundation of coastal habitats is thought to be the reason behind the decline or local extinction of this species in the Nancowry group.

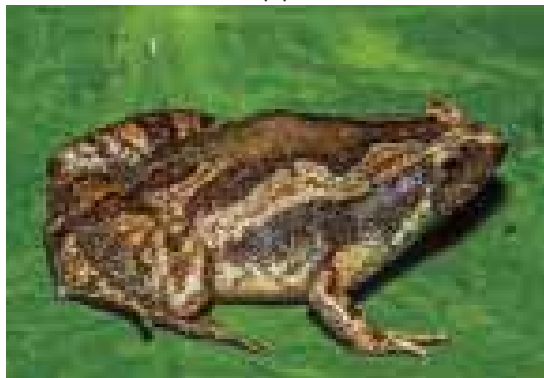
Figure 3: Seven endemic amphibians of Andaman & Nicobar Islands and one potential endemic: a) *Blythophryne beryet*, b) *Kaloula baleata ghoshi*, c) *Microhyla chakrapanii*, d) *Ingerana charlesdarwini*, e) *Fejervarya* cf. *andamanensis*, f) *Fejervarya* cf. *nicobariensis*, g) *Polypedates insularis*, h) *Chalcorana* cf. *chalconota* (originally reported as the subspecies '*raniceps*', its taxonomic status needs further study).



(a)



(b)



(c)



(d)



(e)



(f)



(g)



(h)

Species	Density/ha	Distribution in A&N	Range size	Habitat specificity	IUCN status	WPA (1972)
<i>Duttaphrynus melanostictus</i>	17.8±65.0, 6.4±3	21 (A&N)	Wide	Broad	LC	NL
<i>Blythophryne beryet</i>	51.1±127.3	5 (AND)	Narrow	Specific	NA	NL
<i>Kaloula baleata ghoshi</i>	2.2±14.9	6 (AND)	Narrow	Broad	NA	NL
<i>Microhyla chakrapanii</i>	11.1±38.3	7 (AND)	Narrow	Broad	DD	NL
<i>Microhyla ornata</i>	-	1 (AND)	Wide	-	LC	NL
<i>Micryletta inornata</i>	-	1 (AND)	Wide	-	LC	NL
<i>Microhyla heymonsi</i>	12.5±34.2	2 (NIC)	Wide	Broad	LC	NL
<i>Fejervarya cf. andamanensis</i>	2.2±14.9	3 (AND)	Narrow	Broad	LC	NL
<i>Fejervarya cf. nicobariensis</i>	31.3±87.3	6 (NIC)	Narrow	Specific	EN	NL
<i>Fejervarya cancrivora</i>	2.2±14.9	4 (A&N)	Wide	Broad	LC	NL
<i>Ingerana charlesdarwini</i>	128.9±277.7	10 (AND)	Narrow	Specific	CE	NL
<i>Limnonectes shompenorum</i>	12.5±34.2	4 (NIC)	Narrow	Specific	LC	NL
<i>Limnonectes</i> sp.	25.1±57.7	1 (NIC)	Narrow	Specific	NA	NL
<i>Hoplobatrachus tigerinus</i>	-	3 (AND)	Wide	Broad	LC	Sch IV
<i>Limnonectes doriae</i>	455.6±913.2	10 (AND)	Wide	Broad	DD	NL
<i>Hylarana erythraea</i>	-	7 (NIC)	Wide	Specific	LC	NL
<i>Amnirana nicobariensis</i>	125.1±161.3	9 (NIC)	Wide	Broad	LC	NL
<i>Chalcorana cf. chalconota</i>	-	1 (NIC)	Wide	Specific	LC	NL
<i>Polypedates insularis</i>	-	3 (NIC)	Narrow	Specific	EN	NL

Probably the most important cause of species extinctions in island habitats is invasive species. Ali (2006; 2003; 2004) first drew attention to the problem of invasive species in the Andaman Islands, by listing several such species and demonstrated the negative impact of chital (*Axis axis*) and elephants (*Elephas maximus*) on natural vegetation in those islands where they occur. During our studies, we noticed that islands that had maximum signs of chital (pellets) had lowest abundances of understorey herpetofauna, while the only major island devoid of chital, i.e. Little Andaman Island, had highest density of understorey herpetofauna (Mohanty et al. 2016). Such reduction in abundance of herpetofauna due to the presence of introduced herbivores has also been reported elsewhere (Knox et al. 2012). A recent study found that chital drastically reduced the understorey vegetation in many small islands in the Andaman Islands, reducing the habitat quality leading to reduction in abundance of forest floor herpetofauna (Mohanty et al. 2016).

Indian bullfrogs (*Hoplobatrachus tigerinus*) seem to be recently introduced into the

Andaman Islands, perhaps deliberately for consumption. We found them to be abundant in parts of Mayabunder, Middle Andaman (Harikrishnan & Vasudevan 2013). Though Whitaker (1978) listed this species in a checklist of herpetofauna from the Andaman Islands, there were no further reports and none from other areas. We also recorded this species from South Andaman and North Andaman where it seems to have spread recently. This species has the potential to become a problematic invasive in the Andaman Islands. Its large size could enable this species to competitively exclude and predate on other frog species in the Andaman Islands. It is a prolific breeder and the population could increase rapidly. This species breeds in the beginning of the monsoon and the tadpoles are carnivorous, feeding on tadpoles of other species of frogs (Khan 1996). In mainland India, it is also known to feed on small snakes, lizards and rodents, all of which have numerous endemic species in these islands (Das 1999). It is a species protected by Wildlife (Protection) Act, 1972, Schedule IV Part II.

Table 3: Amphibians of the Andaman & Nicobar Archipelago, with an assessment of their conservation status.

Taxonomic problems:

Three species of frogs, which have been reported from these islands, were not recorded during our surveys. *Limnonectes hascheanus* has been reported to occur in the Andaman & Nicobar Islands (Das 1999). However, Inger & Stuart (2010) in a review of this species and of *Limnonectes limborgi*, provided evidence that the former is restricted to southern parts of Malay Peninsula and expressed doubts on its occurrence in the Andaman & Nicobar Islands. We have not yet recorded any species that matched the redescription provided by Inger & Stuart (2010). *Microhyla ornata* and *Micryletta inornata* were also not recorded during this study and their presence in these islands require further confirmation.

One of the major obstacles for conducting population and community studies of amphibians in the Andaman & Nicobar Islands is the lack of taxonomic clarity for many species, making field identifications difficult and tentative. The small Dicroglossid frogs from the Andaman & Nicobar Islands present several taxonomic issues. The status of species such as *Fejervarya andamanensis*, *Fejervarya nicobariensis*, *Limnonectes hascheanus*, and *Limnonectes doriae* require further taxonomic studies. Another example is the species identified as *Chalcorana* cf. *chalconota* from Great Nicobar Island. Recent phylogenetic studies on this group of forest frogs indicated that they contained multiple, cryptic species in southeast Asia, and several island populations were identified as valid species (Oliver et al. 2015; Inger et al. 2009; Stuart et al. 2006). Das (1996a) reported *Chalcorana chalconota* (Schlegel 1837) (as *Rana chalconota*) from Great Nicobar Island, while mentioning that the individuals had diagnostic characters of the subspecies *R. chalconota raniceps* (Peters 1871). *Chalcorana raniceps* (Peters 1871) is currently considered as a valid species, but endemic to the island of Borneo (Inger et al. 2009). In their review of the systematic of frogs previously assigned to the species *C. chalconota*, Inger et al. (2009) restricted the range of *C. chalconota* sensu stricto to Java and South Sumatra, while also describing two new species, including

C. rufipes (Inger et al. 2009) from Western Sumatra. Frost (2017) lists the population from Great Nicobar as *C. labialis* (Boulenger, 1887), a species occurring in peninsular Malaysia. However, the population from Great Nicobar was not part of any of the recent reviews of this group, and it is impossible to assign this population to any of the currently recognized species.

Conclusion

The Andaman & Nicobar Islands contain a small but unique amphibian fauna, with the potential for many more species to be recognized. They are also vulnerable to extinction due to their small distributional ranges and dependency on fresh water in island systems. Since the Andaman Islands are also a major tourism destination, spread of infectious diseases, such as Chytridomycosis that is considered responsible for the greatest disease-caused loss of biodiversity in recorded history, is also a potential threat to amphibians these islands (Skerratt et al. 2007). To create effective monitoring and conservation programmes for amphibian species in these islands, it is necessary to clarify the taxonomic issues regarding the identities of species in these islands.

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Amphibians of Sikkim Himalaya: An evaluation of diversity, distribution pattern and threats along the elevation gradient

Abstract

Amphibians constitute an important component of ecosystem linking minor vertebrates with major and aquatic life with terrestrial. Unlike birds and mammals, the study of amphibians is mostly limited to taxonomy. Understanding the diversity and distribution of species along the geographical gradient will aid in conservation and management of biodiversity. With the projected impact of climate change in the Himalayas, the study of distribution pattern along the elevation gradient is very important. Sikkim, part of the Eastern Himalayan Biodiversity Hotspots, exhibits immense gradient of altitude and climate resulting in great variation of life forms. Amphibians were studied along the elevation gradient of Sikkim Himalaya. A total of 28 species was recorded during the study period with maximum richness between 1000-2000m elevations. Most amphibian species of Sikkim Himalaya were confined to narrow elevational width. Hence conservation of entire gradient of habitat is essential for the conservation of amphibians. During our study, we found extraction of frogs by locals for meat and medicine. With the rapid land use change, climate change and unsustainable harvesting, the amphibian of the Himalaya poses great challenge for future survival.

Introduction

Amphibians play an important role in energy flow and nutrient cycling in the ecosystem and serve as the bio-indicators of ecosystem health because of the sensitive permeable skin and dual mode of life (Beebee and Griffiths, 2005). Amphibian populations are declining and disappearing globally at a fast rate even from protected areas (Blaustein and Wake, 1990; Stuart et al. 2004). However, new species are continuously being discovered at the high rate (25% in the last 11 years). Many new species are being continuously described from North East India (Kamei et al. 2009; Biju et al. 2010; Mahony et al. 2013; Subba et al. 2015; Biju, 2016).

Rodgers and Panwar (1988) included Sikkim as part of Trans Himalaya and Central Himalayas. However, other literatures considered the state as part of the Eastern Himalayan region (Ali, 1962; Mani, 1974). Sikkim is now a part of two biodiversity

hotspots, Indo-Burma hotspots and Himalaya hotspots (Mittermeier et al. 2004). Although Sikkim is only one twentieth of the Western Ghats in geographical area, diversity of flora with 4,458 species of flowering plants including 525 species of orchids, 58 primulas and 36 rhododendrons is spectacular. Fauna also displays rich diversity with 169 species of mammals, 574 birds, 88 reptiles (71 snakes and 17 lizards), 50 amphibians, 50 fishes and 689 species of butterflies (Acharya and Sharma, 2013). This high diversity is attributed to its geographical location on the confluence

Key words:

Amphibian, Conservation, Elevational gradient, Extraction, Himalaya, Sikkim

Nasutixalus jerdonii.
Photo Credit: Abhijit Das

Basundhara Chettri

Department of Zoology, Sikkim University
Barad Sadan, 5th Mile, Tadong - 737102, Gangtok, Sikkim.

Corresponding author e-mail: bchettri01@cus.ac.in



between Oriental and Palearctic zoogeographical realms, an elevation range extending from 300 m to over 8598 m and climatic regime from tropical to cold desert. Despite high diversity, Northeast India is far less explored as compared to Western Ghats and much of the diversity awaits scientific discovery (Biju et al. 2016).

Diversity and distribution of life forms along the geographical gradients such as latitude and elevation has fascinated biogeographers since past many years. Why species declines from tropics towards the poles? Similarly, why some species are restricted to sea level while others at the mountain tops? Many studies around the globe have attempted to understand these uneven patterns of distribution, yet the generality of such trend is unknown. The study of species distribution pattern along the different elevational gradient will help in prioritization of areas for conservation of biodiversity (Gaston, 2000; Hu et al. 2011; Aynekulu et al. 2012). Apart from biodiversity conservation, such studies are crucial for evaluating the impact of climate change under which contraction or expansion of species range size are expected (Ah-Peng et al. 2012).

The people in the Himalayan region, including Sikkim live in close association with nature. The livelihood of Himalayan people revolves around the natural resources but with current rate of population increase and anthropogenic activities taking place, biodiversity of this region is in serious threat (Schleich & Kästle, 2002). We noticed that local and migrant communities exploit various species of amphibians for meat and medicine endangering the species pool in the region. While the local communities of Sikkim Himalaya especially aboriginal tribe "Lepcha" have profound indigenous knowledge on biodiversity which can be utilized for the conservation of rich biodiversity of the region (Acharya et al. 2009).

Here, I compiled and updated the checklist of amphibians of Sikkim based on extensive research and available literatures. The elevational range of the amphibian species are obtained from both primary and secondary data. The diversity and distribution pattern of amphibians along the



elevation gradient are evaluated to identify diverse areas of conservation importance. In addition, threats to amphibians with reference to extraction by humans were also evaluated. Certain conservation measures have been recommended.

Ingerana borealis.
Photo Credit: Abhijit Das

Study Area

Sikkim, one of the smallest states of India (27° 5' - 28° 10' N and 87° 4' - 88° 58' E) is situated at the western most boundary of eastern Himalayas and encompasses geographical area of 7096 km². Entire Sikkim is considered as the catchment of River Teesta. Sikkim has 180 perennial lakes among them Khechopelri, Guru Dongmar, Chho-Lhamu, Tsomgo and Menen Tso are a few high altitude lakes to mention, which are not only of scenic and religious importance, but have immense ecological value (Acharya and Sharma, 2013). The catchment area along the course of the river is wet and humid, conducive for the amphibians. Besides, the swift tributaries of Teesta shelter many torrent frogs, which are either unknown or poorly known.

Within a very small geographical span (linear distance of ~150 km), climate changes from hot tropical to temperate condition, vegetation from tropical semi-deciduous in the lower elevations through temperate broad-leaved forest at the mid and coniferous to sub-alpine and alpine vegetation at the higher altitude. The other abiotic factors such as humidity, rainfall, temperature and atmospheric pressure also changes accordingly. Lower valleys are hot and humid and receive heavy rainfall, sometimes exceeding 3500mm per annum (Chettri et al. 2010). The high elevation

regions are drier and colder with relatively less annual precipitation. Precipitation in the sub-alpine and alpine region occurs in the form of snow.

In addition to literature survey, an extensive field research on amphibians was carried out in the Teesta valley and Maenam Wildlife Sanctuary in Sikkim during 2009-2010. Teesta originates at snow fed Chollamu Lake, at 4800m above mean sea level and flows in north-south direction till it

enters into the West Bengal near Melli at 300m. Survey locations for amphibians are shown in Figure 1. For data collection, study area was categorized into seven zones based on elevation (500m interval; Table 1). Apart from extensive study in Teesta valley, surveys were carried out in Maenam Wildlife Sanctuary, located on the Tendong-Maenam ridge within the southern district of Sikkim and spreads over a geographical area of 36 sq km (Fig. 1).

Table 1: Categorization of the study area based on altitude (500m interval) in Sikkim

Elevational zone	Elevation (m)	Forest type
Zone I	< 500	Tropical Semi Deciduous Forest (TrSDF)
Zone II	500-1000	Tropical Semi Deciduous Forest (TrSDF)
Zone III	1000-1500	Tropical Broad Leaved Forest (TrBLF)
Zone IV	1500-2000	Tropical + Temperate Broad-Leaved Forest (TrBLF + TmBLF)
Zone V	2000-2500	Temperate Broad-Leaved Forest (TmBLF)
Zone VI	2500-3000	Temperate Broad-Leaved + Coniferous Forest (TmBLF + CnF)
Zone VII	>3000	Coniferous Forest + Sub-alpine + Alpine (CnF + SAl + Al)

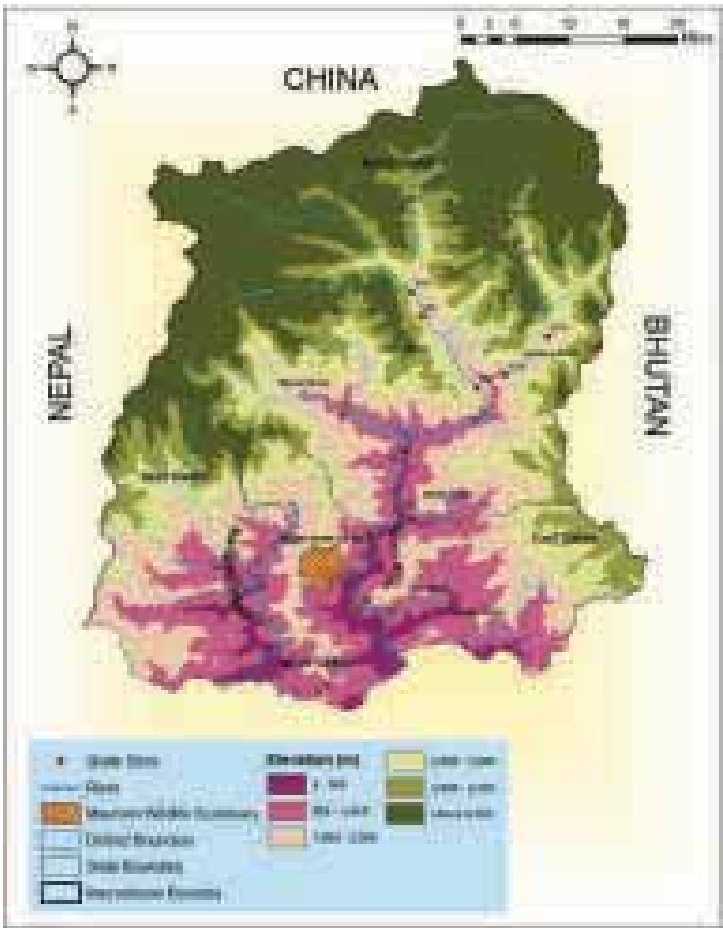


Figure 1: Map showing the sampling locations in Teesta valley (red dots) and Maenam Wildlife Sanctuary in Sikkim. (S1 - Melli; S2 - Singtam; S3 - Reshap Dalep; S4 - Dikchu; S5 - Sangkalang Dzongu; S6 - Toong; S7 - Muyong; S8 - Chungthang; S9 - Lachen; S10 - Zema; S11 - Thangu; S12 - Khedum; S13 - Lachung; S14 - Yumgthang; S15 - Yumesamdong (South)).

MATERIALS AND METHODS

Compilation

Thorough literature review dealing with herpetofauna of Sikkim and neighbouring areas of Darjeeling Hills was undertaken to generate information on species occurrence in the state. Data on elevational range were also obtained from literature supported by the field observations.

Data collection

Field study was carried during April 2009–July 2010 with intensive sampling during May to August when species are breeding and, therefore, activity is at its highest. Time constraint visual encounter survey (VES) method (Heyer et al. 1994) was followed for sampling amphibians. Search was conducted during day hours (09:00 to 14:00 Hrs) and microhabitats such as boulders, logs and mosses were thoroughly examined searching for the animals. Since most amphibians are crepuscular or nocturnal, VES was supplemented by night stream surveys (19:00 to 20:30 Hrs) in various locations. Data on type and extent of any kind of disturbance(s) were noted to assess the threat on amphibians. Total number of people involved in extraction, community type (such as Lepcha, Bhutia, Rai, Limboo, Tamang, etc.), location and elevation, species and number of individuals caught were collected. The persons involved in such activities were casually interviewed to verify the data and to gather more information.

Data analysis

The data generated during the study were analyzed using different computer packages and statistical software such as SPSS and Microsoft excel. Species richness, diversity, evenness and abundance were calculated for total and each elevation zone separately. Species richness was obtained as cumulative number of species and abundance as total number of individuals observed during the study. Based on these data, we estimated distribution pattern along the elevation gradient. Anthropogenic threat on amphibians was analyzed based on extraction to figure out the total amphibian extraction per site, preferred species, purpose of extraction and time of collection.

Results

Species richness and composition

Number of species reported from Sikkim by different researchers varied from 15 to 30. Boulenger (1890) and Gammie (1928) reported 17 and 16 species of amphibians respectively from Sikkim. Inger and Dutta (1986) summarized amphibian distribution based on the literature and reported 56 species from Northeast India, 15 specific to Sikkim. Waltner (1973) documented 53 species of amphibians from the entire range of Himalayas including 30 from Sikkim. Swan (1993) found 41 amphibian species from plains and mountains of both Sikkim and Darjeeling area, but reduced the species confined to hills as 32. Chanda (2002) documented Indian amphibians, which included 21 species found in Sikkim. Jha and Thapa (2002) reported 26 species of amphibians from Sikkim Himalayas of which 20 are specific to Sikkim. Most of the above reports are based on museum records and compilation from published literature except the recent work by Subba et al. (2016) which reported the occurrence of 23 amphibian species from Sikkim Himalaya.

Based on various published literatures (Boulenger, 1890; Chanda, 1986; Schleich & Kastle, 2002, Jha and Thapa 2002; (Subba *et al.* 2016) and the field study conducted in Teesta valley and Maenam Wildlife Sanctuary we found occurrence of 37 amphibian species (Table 2). Of the total 37 species, 35 species belong to five families under order anura and the remaining two species are represented by each order urodela and gymnophiona. Out of total 37 species, nine are not evaluated (NE) and

Nanorana cf. annandalii.
Photo Credit: Abhijit Das



two are data deficient (DD) hence their population status in the wild is not known.

Field study in Teesta valley and Maenam Wildlife Sanctuary yielded 25 amphibian species. These species belonged to seven families and two amphibian orders (Table 2). Order Apoda was represented by single

family and species, whereas order Anura comprised of six families and 24 species. According to IUCN red list one species is under vulnerable (VU) category (*Ingerana borealis*) and two under near threatened (NT) category (*Nanorana gammii*, *Nanorana annandali*, *Nanorana ercepeae* and *Rhacophorus reinwardtii*).

Amphibia Family	Species	Elevation range(m)	IUCN Red list categories
Order - Anura			
Family			
Bufonidae	<i>Duttaphrynus himalayanus</i> *	1000-3500	L C
	<i>Duttaphrynus melanostictus</i> *	300-1800	L C
	<i>Duttaphrynus stuarti</i> ⁵	500-650	NE
	<i>Duttaphrynus stomaticus</i> ³	300-1800	NE
Megophryidae	<i>Megophrys major</i> ¹	300-2500	NE
	<i>Megophrys parva</i> *	1500-2300	NE
	<i>Megophrys robusta</i> *	800-2300	NE
	<i>Scutiger boulengeri</i> *	4005-5270	L C
	<i>Scutiger sikkimensis</i> *	2619-4395	LC
Dicroglossidae	<i>Fejervarya nepalensis</i> *	1350-1580	L C
	<i>Fejervarya teraiensis</i> *	300--700	L C
	<i>Hoplobatrachus tigrinus</i> *	300-500	L C
	<i>Nanorana annandalii</i> *	1500-2650	NT
	<i>Nanorana blanfordii</i> *	1400-2000	L C
	<i>Nanorana ercepeae</i> *	2200-2600	NT
	<i>Nanorana gammii</i> *	1000-2000	NT
	<i>Nanorana liebigii</i> *	1220-3000	L C
	<i>Nanorana polunini</i> *	2300-3390	L C
	<i>Ingerana borealis</i> *	400-1000	VU
Ranidae	<i>Ombrana sikkimensis</i> ⁴	1200-2500	LC
	<i>Amolops marmoratus</i> *	1000-2165	L C
	<i>Amolops monticola</i> *	1060-2350	L C
	<i>Amolops formosus</i> *	1190-2480	L C
	<i>Amolops gerbillus</i> ⁴	300-1700	L C
	<i>Amolops himalayanus</i> *	1700-2300	L C
	<i>Clinotarsus alticola</i> ⁴	900	L C
Rhacophoridae	<i>Odorana livida</i> ⁴	300-1000	NE
	<i>Polypedates leucomystax</i> *	300-1500	L C
	<i>Polypedates maculates</i> *	300-2400	NE
	<i>Polypedates megacephalus</i> *	300-1500	L C
	<i>Raorchestes annandalii</i> *	900-2700	L C
	<i>Philautus dubius</i> ²	1000-1500	DD
	<i>Nasutixalus jerdonii</i> ²	1500-2000	NE
	<i>Rhacophorus maximus</i> ⁵	500-2000	L C
Order - Gymnophiona			
Ichthyophidae	<i>Ichthyophis sikkimensis</i> *	1550-2000	DD
Order - Urodela			
Salamandridae	<i>Tylototriton verrucosus</i> ¹	1200-3350	LC

LC: Least concern; NE: Not evaluated; NT: Near threatened; VU: Vulnerable; DD: Data deficient.

Table 2: Checklist of Amphibians (38 species) of Sikkim and their altitudinal distribution (Source: 1-Boulenger, 1908; 2-Waltner - 1973; 3-Schleich & Kastle, 2002; 4-Mathew and Sen, 2010; 5-Subba et al. 2016). * indicate the species recorded during the present study

Species abundance

Relative abundance of amphibian species of Sikkim Himalaya based on field study shows sparse distribution. Species abundance model showed steep decline in abundance pattern with few species dominating the amphibian community. This pattern was consistent for each zone separately as well as for the whole study area. Single species *Duttaphrynus himalayanus* represented ~600 individuals while another two species showed more than 100 individuals. Four species showed ~50 individuals while remaining 18 species had less than 50 individuals. The abundance distribution model clearly indicates that in an amphibian community a few species were common and most of them rare (Fig. 2).

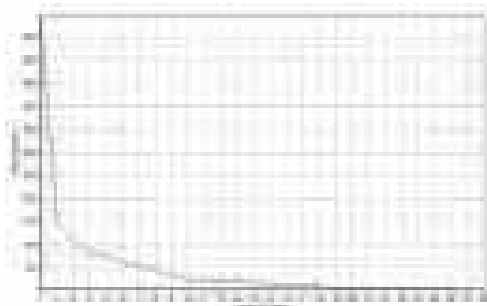


Figure 3: Species abundance model of amphibian population in Sikkim Himalaya.

The species abundance data for amphibian of Sikkim Himalaya fits to truncated log normal distribution (Fig. 3). There was no significant difference in observed and expected number of species in each abundance category ($\chi^2 = 1.43$; $p = 0.92$; $df = 5$).

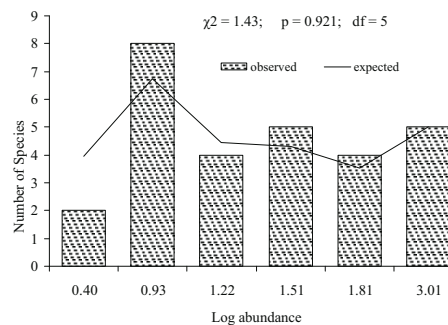
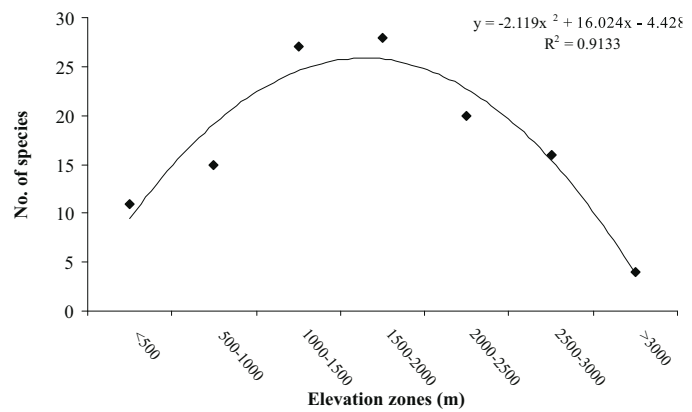


Figure 4: Species abundance data of amphibians of Sikkim Himalaya fits to truncated log normal distribution.

Elevational distribution pattern

Amphibian species richness when plotted against elevation based on compiled data (37 species) showed unimodal pattern. The species richness peaked at 1000-2000m elevations with tapering ends towards both sides though the decline was sharp towards the higher elevation (Fig. 4). Only four species were present above 3000m elevation.



Field study along the Teesta valley and Maenam Wildlife Sanctuary also revealed similar elevational distribution pattern with peak between 1000-2000m (Fig. 5; $R^2 = 0.86$; $p = 0.01$). Maximum of 16 species were observed in zone IV (1500-2000m) followed by 14 species in zone III (1000-1500m). Only three species were found in highest elevation zone (>3000m) showing four-fold decline in species from mid to high elevation zone. Areas between 1000-2000m includes such as Sangkalang, Toong, Theeng, Chungthang, Lingee, Upper Payong, Maenam Wildlife Sanctuary. It is also to be noted that many developmental activities are now initiated in these areas which may degrade the probable habitat of many species which are yet to be explored. Abundance showed different pattern along the elevation gradient. The abundance peaked at 2000-2500m followed by 2500-3000m.

Figure 4: Elevational distribution pattern of amphibian species in Sikkim Himalaya (compiled data).

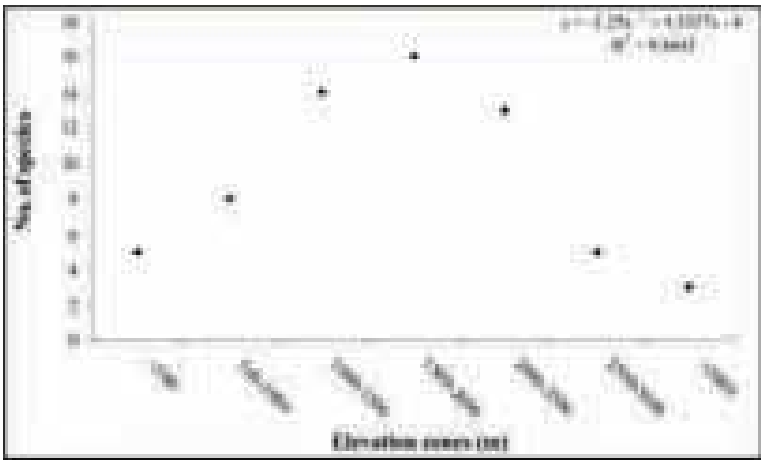


Figure 5: Elevational distribution pattern of amphibian species in Sikkim (present study).

Elevational Range Size

Compilation of range size of amphibian species of Sikkim Himalaya from various sources indicates that none of the species occur along the entire gradient of elevation. Out of total 37 species, only four species had more than 2000m elevational range and 10 species had less than or equal to 500m elevation width (Fig. 6). Both the lower and higher elevation species extended their range towards middle elevation thus

increasing the species richness there. Present study in Sikkim Himalaya indicates that most species had narrow elevational range size (Fig. 7). Out of 25 species, 7 species showed very narrow elevational width (<500m) and single species i.e. *Duttaphrynus himalayanus* had broad elevational width from 1100m to 3300m. Elevational range of most amphibian species of the present study was reduced as compared to compiled data (Fig. 6 & Fig. 7).

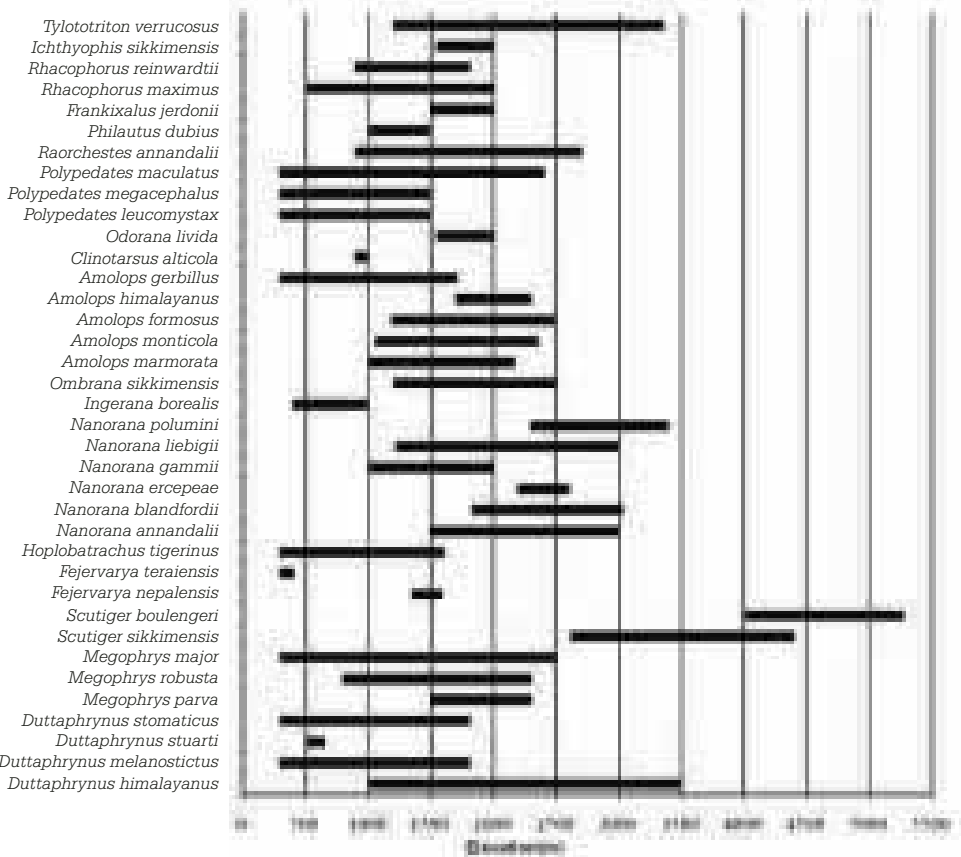


Figure 6: Elevational range profile of amphibians of Sikkim Himalaya based on compiled data.

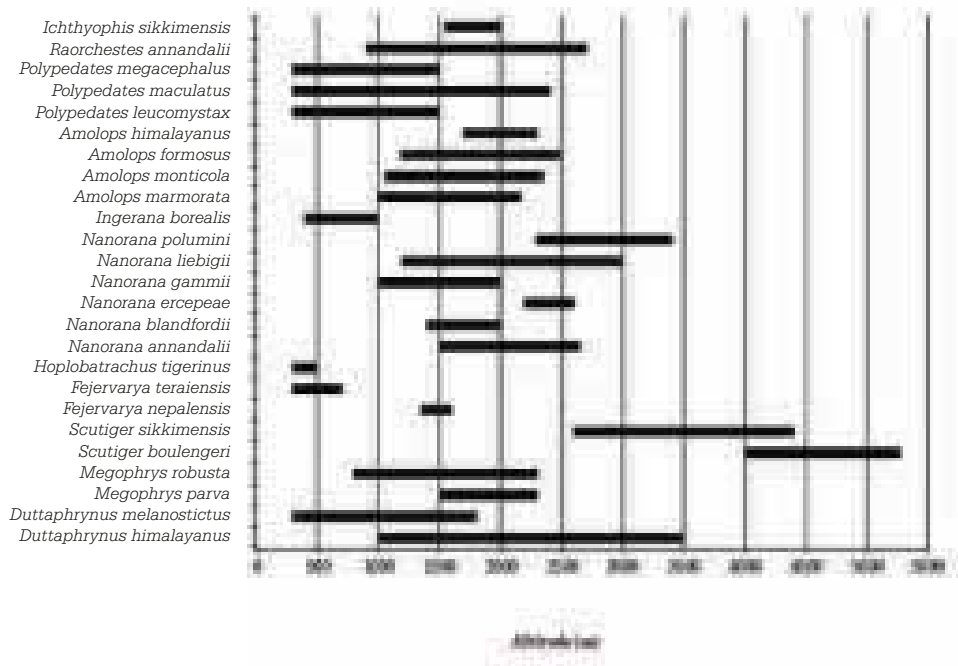


Figure 7: Elevational range profile of amphibians of Sikkim Himalaya based on present study.

Species similarity among elevation zones

Species similarity among elevation zone was generally low reflecting potential of each zone in harbouring unique amphibian assemblage (Fig. 8). Among the pair of zones, maximum similarity (0.8) was observed between 2500-3000m and >3000m elevation zones. However >3000m stands distinct from the rest of the elevation zones forming separate assemblages. Species similarity index among pair of zones depicts distinct community composition in each zone.

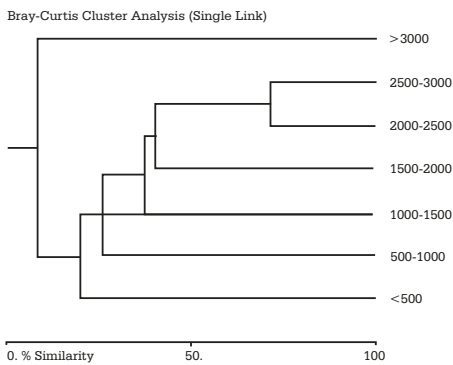


Figure 8. Dendrogram showing similarity of species composition among elevation zones in Sikkim Himalaya.

Discussion

Species richness and composition

Eastern Himalaya part of two biodiversity hotspots, the Indo-Burma and Himalaya Hotspots, possess great diversity of flora and fauna. The high diversity is attributed to its vast gradient of altitude reflecting similar gradient of climate and vegetation zones. Sikkim, part of Eastern Himalaya, represents high diversity of amphibian fauna and many new species are awaiting proper documentation. Amphibian species of this region is morphologically cryptic and remains significantly underestimated. Due to cryptic diversity, fourteen species has been described from two species in South-east Asia (Stuart et al. 2006). The taxonomy of amphibian is further complicated by many mis-identified amphibians such as *Megophrys boettgeri*. It was retained in the Indian list of amphibians because the tadpole of this species was similar to *Megalophrys kempii* of Arunachal Pradesh disregarding the clear morphological differences between the adult of both species (Mahony et al. 2013).

Polypedates leucomystax and *Polypedates maculates* have been reported from Sikkim by various authors including recent study by Subba et al. (2016). However, identification

of the species is not clear yet. Identification keys provided by authors such as Deuti and Goswami (1995) and Subba et al. (2016) seem different for these species. Further, *Polypedates himalayanus* and *Polypedates megacephalus* possess taxonomic confusion. Similarly, *Duttaphrynus* groups are largely underestimated and wide variation occurs along the altitude gradient. Species like *Duttaphrynus stomaticus* might have been overlooked as *Duttaphrynus melanostictus*. The present study could not locate Himalayan Salamander (*Tylototriton* sp.) in Sikkim though many literatures reported its occurrence in Sikkim (Boulenger, 1890; Gammie, 1928; Mathew and Sen, 2010). Prior to Indian independence, Sikkim was a separate Kingdom including many hills and terai regions of Darjeeling known as greater Sikkim. Hence, many authors include the species in Sikkim list. However, even the recent literature supports its occurrence in Sikkim. It is probably due to the proximity and the similarity of the habitat of Sikkim with that of Darjeeling where *Tylototriton* sp. is found. During a span of more than 10 years of our research work in Sikkim Himalaya we could not find it. However, it is also to be mentioned here that our research was focused along the Teesta basin in north and south district of Sikkim. Intensive research in probable habitat in west and east district will help to draw final conclusion on its occurrence in Sikkim Himalaya.

The number of amphibian species reported by various authors varied from 15-30. Variation in the number of species reported by different authors could be probably due to the reason that during pre-independence, Darjeeling Hills and adjacent plains were considered as part of greater Sikkim Himalayas (Gammie, 1928) and inclusion of species found in Darjeeling area might have resulted in this inconsistency. Other reasons such as taxonomic confusion and frequent changes in nomenclature in amphibian taxonomy and unequal sampling bouts/observation periods could have led to these differences.

In 2015, one of the high elevation frog *Scutiger boulengeri* was added to the list of amphibians of Sikkim at an altitude of 5270m with the use of molecular and



Amolops formosus.
Photo Credit: Abhijit Das

morphological data (Subba et al. 2016). Recently, two new species (*Duttaphrynus stuarti* and *Fejervarya nepalensis*) was again added to the list of amphibians of Sikkim by Subba et al. (2015). More scientific studies and explorations are necessary to generate the complete list of amphibians of Sikkim.

Based on previous publication, we compiled a list of 37 amphibian species with a record of occurrence in Sikkim Himalaya. The present study along the Teesta Valley and Maenam Wildlife Sanctuary yielded 25 amphibian species. Representing such high diversity, the study area serves as potential habitat very conducive for amphibians. The high diversity in comparison to small area is attributed to high variation in altitude (300 - 5000m), climatic condition (Tropical - Arctic) and occurrence of streams and rivulets (tributaries to Teesta River) along the valley. Out of 37 species reported to occur in Sikkim, nine are not evaluated or two data deficient and hence their population trend and threat status is not known. The study on these taxa therefore should be taken up in priority to understand the distribution pattern, population trend and potential threats in order to implement any kind of conservation measures. Additionally, while planning and management of biodiversity conservation, equal justice should be given to amphibians so that protected areas and reserves includes some of these amphibian hotspot regions.

Northeast India comprising of Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, Sikkim and Darjeeling district of West Bengal harbours

very high diversity and endemism of amphibian fauna. In the recent past many new amphibians have been described from the region (Kamei et al. 2009; Biju et al. 2010; Mahony et al. 2013; Subba et al. 2015; Biju, 2016). Occupying just 2.8% geographical area of Northeast India (about 255,168 sq km), Sikkim harbours 37% of species. The data reflects high diversity of amphibians in Sikkim relative to its small geographical extent. With more research the numbers of amphibian species are likely to increase.

Species abundance model

Amphibian abundance data showed that most of the species are rare and very few are common. This is indicative of more diverse ecosystem, especially tropical forests (Heatwole, 1982; Magurran, 1988). Species abundance data of amphibians fits to truncated lognormal distribution which signifies the stability and equitability of the amphibian community. No significant difference between observed and expected abundance indicate that our sampling encountered most of the individual occurring in the study area.

Elevational distribution pattern

The elevational pattern of amphibians in Sikkim followed mid-elevation peak (both compiled and field based data). The pattern is consistent with birds and plants (Acharya et al. 2011a; 2011b) but slightly different from reptiles (Chettri et al. 2010) from the same study area. While few studies have reported monotonic decline of reptiles and amphibians (Heatwole, 1982; Khatiwada and Haugaasen, 2015), mid-elevation peak is the most commonly observed pattern of amphibian species richness (Fu et al. 2006; Hu et al. 2011; Araujo et al. 2015). In contrast, the anuran study along the altitude gradient of Western Ghats, South India reported increasing trend with elevation (Naniwadekar and Vasudevan, 2007). Growing evidences supports the mid-elevation peak for amphibians whereas few diversions from this pattern occurred where the sampling did not cover the entire elevation gradient available (Naniwadekar and Vasudevan, 2007; Khatiwada and Haugaasen, 2015). The variation of pattern among taxa is mainly due to the differences in their physiological requirement and micro

habitat preference. For amphibians and reptiles, temperature, precipitation and relative humidity plays significant role (Chettri, 2010; Chettri et al. 2010) whereas for birds and plants factors such as productivity and habitat heterogeneity was significant (Acharya et al. 2011a, b). Climatic condition is the most determining factor for the species distribution especially for the ectotherms (Fu et al. 2006).

Abundance showed different pattern along the elevation. High abundance towards higher elevation was due to the clumped distribution of single species *Duttaphrynus himalayanus*. Increased abundance with elevation is due to the proliferation of few species as species richness declined towards higher elevation (Heatwole, 1982).

Elevational Range Size

The narrow elevational range of most amphibian species reflects their sensitivity towards various environmental factors which changes at a faster rate along the elevation gradient. The range sizes have been used as a predictor of extinctions; narrow range species have greater risk of extinctions (Harris and Pimm, 2008; Hu et al. 2011) and are of special interest for the conservation of biodiversity (Ah-Peng et al. 2012). Most amphibians of Sikkim Himalayan had narrow elevational width with many species confined to less than 500m. Most species are restricted to single zone and unable to extend their habitat beyond certain limit. Hence, the conservation of habitats along the entire elevational gradient is necessary as many

Microhyla cf. ornata.
Photo Credit: Abhijit Das



species are restricted within narrow range.

The comparison of range size between present data and compiled data showed slight variation. Since the compilation is based on various sources, species showed broader elevational range than the actual range they may occupy. Further, amphibians in Western Himalaya are found in higher elevation than in Eastern Himalaya (Schleich & Kastle, 2002). However, the amphibian in Sikkim Himalaya occupy narrow elevational range and any disturbance might lead into the decline of the population.

Species similarity among the elevation gradient

Similarity among elevation zones was low except between 2500-3000m and >3000m. Maximum similarity between 2500-3000m and >3000m elevation zones is due to the similar climatic and habitat conditions. The distinct amphibian assemblage >3000m may be due to abrupt change in climatic condition and vegetation types (broadleaved to coniferous forest) which acts as barrier for species dispersal (Gainsbury and Colli, 2003). It could be also due to isolation mechanism (Lomolino, 2001). The snow covered mountain peaks with steep slopes and circulating cold winds would have resulted in the isolation of species in the present case. The low similarity indicates that all zones contain unique assemblage of species and are distinct from other zones. Despite low richness in some zones, the conservation of representative habitats of all zones is necessary for the holistic conservation of amphibians.

Anthropogenic Threats

Extraction

We observed that local people rampantly collect amphibians from various streams. The collections are used as meat supplement as well as for some medicinal purposes after smoke dry (Fig. 9). Amphibian collection is done mostly during dusk to early night hours (19:00-21:30 hours). The people involved in extraction of amphibians during night use locally made kerosene lamp known as pultho and collect whatever they encounter. Occasionally, collections during day hours were also observed.

Based on surveys in different places along the Teesta valley such as Dzongu, Pabong, Lingee, Chungthang and Lachung and Maenam Wildlife Sanctuary, it is estimated that one individual (which most often represents one household) collects 30-35 individuals per night in an average. The collection is intense during monsoon season (June - August) with an approximate collection frequency of three times a week per household. The most preferred species are *Amolops* spp., *Nanorana* spp. and *Xenophrys* spp., although all kinds of frogs are hunted (Fig. 9). *Nanorana* species is locally known as Paa and is extensively used for medicinal purposes. Most of these species occur in the middle elevation and maximum extraction hence also occurs in mid-elevation where the maximum diversity occurs.

Besides ecological and cultural values, frogs constitute important source of protein for economically unstable communities (Gonwouo and Rodel, 2008). In Sikkim, the floating communities such as personnel working as labourers in hydro power projects and road construction activities along with few ethnic tribes are responsible for mass extraction. Further, extraction coincides the peak breeding period i.e. June to August. The unsustainable harvesting threatens the amphibian population and their habitats specially the restricted ranged species (Hu et al. 2011).

Local communities belonging to Lepcha, Bhutia, Rai and Limboo are involved in extraction mostly for medicinal purpose as well as for dietary supplement. The species preference varies among the communities. Lepcha and Bhutia communities collect almost all types of frogs, whereas other communities confined with webbed feet species locally called 'Paa' such as *Amolops* spp., *Nanorana* spp.

Habitat destruction

Apart from extraction, amphibians of Sikkim Himalaya faced tremendous threat from habitat destruction. During monsoon, the collectors divert water from the stream to other side for fishing. In this process large number of eggs and tadpoles present there gets dehydrated and die. This activity has posed serious threat to amphibian population as well as their habitat (especially breeding) causing population

decline. Another significant threat to amphibian habitat is mining in streams. Anthropogenic disturbance to breeding habitats of amphibians is considered as the most serious threat for population decline worldwide (Mac Nally et al. 2009). Rampant

extraction of sand, gravel and boulders affect the amphibian microhabitat. Hence, regulation of such extraction is necessary for conservation of amphibian fauna and their habitat.



Figure 9: . Frog extraction at Dzongu, North Sikkim by locals during night (A); Frogs kept for smoking above fire after collection (B). The displayed frog comprises of two most extensively hunted species - *Amolops* sp. and *Megophrys* sp.

Conclusions and Recommendations

Amphibian, and also birds and plants, shows highest diversity at mid-elevation in Sikkim Himalaya (Chettri, 2010; Acharya et al. 2011a; 2011b). Hence, priority should be given for the conservation of the areas in these elevation zones. A landscape conservation approach with community participation is highly recommended.

In Sikkim, though 34% of the geographical area is under protection, most of the protected areas lie above 2000m. Protected areas notified based on larger vertebrates may not do equal justice to minor vertebrates like reptiles and amphibians (Vasudevan et al. 2006). Multi-species study in Hong Kong also found mis-match between species richness and protected areas (Yip et al. 2004). Similar conclusion was drawn by the study of Khan et al. (1997) in Meghalaya. Hence, the present study

strongly recommends extending the protected areas towards lower elevation. Further, while planning the protected areas, minor taxa should also be taken into consideration.

Illegal harvesting of frogs and disturbance to habitats by local community was recorded in the study areas. Though extraction by native tribe for medicinal purposes can be sustainable as they used to do before also but with increasing population and developmental activities leading to habitat destruction, the extraction cannot be sustainable. More awareness about importance of these minor vertebrates in ecosystem can help in reducing this threat to great extent.

Acknowledgements

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Endemic and Threatened Amphibians from the Sacred Groves and Plateaus of Western Ghats in Maharashtra

Abstract

We studied the amphibian diversity and distribution with major emphasis on their endemism and conservation in the six sacred groves and six high altitude plateaus in the Western Ghats of Satara, Pune and Raigad districts of Maharashtra. Although these sites fall outside legal protected areas and are diminutive in size compared to a Sanctuary or National Park however they are patches of native biodiversity rich forests. Importance of such sites in terms of conservation of endemic species of Northern Western Ghats are discussed. These habitats are experiencing land use changes due to infrastructural and anthropogenic development. It is feared that fragmentation or degradation to these sites will cause local or irreversible extinction of endemic species. During our surveys we recorded seven endemic species from seven families of amphibians. Out of which one species is Endangered, two are Vulnerable, three are Data Deficient and one is Not Evaluated on IUCN redlist. This study unveils the fact that most of these points are functioning as refuge to the populations of endemic and threatened amphibians in Western Ghats and their conservation is important.

Introduction

Worldwide acclaimed biodiversity hotspot Western Ghats is the center of amphibian diversity and endemism (Biju 2001; Garg and Biju 2017). In vertebrates group, amphibians exhibit the highest level of endemism in Western Ghats of India. In Cretaceous period, a long topographical separation of Indian subcontinent may have given conditions for high endemism (Van Bocxlaer et al., 2012). The current Indian amphibian species count is ca 429 (Frost 2017) and 413 (Amphibia Web 2017). Several species are at the verge of extinction due to anthropogenic pressure (Biju et al., 2011). There have been many studies in the protected areas such as national parks and tiger reserves however there is a paucity of research data from legally unprotected and community conserved areas such as sacred forest or grasslands especially with respect to amphibian fauna. This study is an attempt to bridge this gap and to discover diversity of endemic amphibians in areas outside legal protection.

Key words:
Amphibians, endemic, Sacred groves, Maharashtra

Eggs of *Nyctibatrachus humayuni* from Maharashtra.
Photo Credit: Abhijit Das

Vishal Kumar Prasad¹*, Rahul Mungikar², Shamita Kumar³, Kranti Yardi³, Bapurao Vishnu Jadhav^{4&5}, Laxmikant K Desai⁶ & Angshuman Baruah¹

¹Wildlife Institute of India, Post Box No. 18, Chandrabani, Dehradun, Uttarakhand, Pin Code- 248001

²Maharashtra State Biodiversity Board, Jaivividhata Bhavan, Forest Colony, Salunke Vihar, Wanowrie, Pune Office, Pune – 411048

³Institute of Environment Education and Research, Bharati Vidyapeeth Deemed University, Katraj, Pune, Maharashtra, India, Pin code - 411043

⁴Department of Zoology, Balasaheb Desai College, Patan, District Satara- 415 206, Maharashtra

⁵Biodiversity Research and Conservation Foundation, 73 Gulmohar Colony, ITII Road, Satara, Maharashtra, Pin code 415002

⁶Environment Conservation and Research Organization, 151 Ambica Nagar, Pale – 403105, Goa

*Corresponding author e-mail: vishalkprasad@wii.gov.in



Since the British colonial era, western Maharashtra has been in focus for amphibian research. Novel species were described since 1888, for example *Indirana leithii* (Boulenger 1888) from Matheran and *Fejervarya syhadrensis* (Annandale 1919) from Satara and Pune districts. After independence, more species such as *Nyctibatrachus humayuni* (Bhaduri and Kripalani 1955) from Mahabaleswar (Satara District), *Indotyphlus battersbyi* (Taylor 1960) from Khandala, Poona District, *Xanthophryne koynayensis* (Soman 1963) from Koyna Satara district were discovered.

Recently, many herpetological searches in high rainfall areas such as Amboli forest (Sindhurg district), Koyna Wildlife Sanctuary (Satara district), Phansad Wildlife Sanctuary (Raigad district), Tahmini (Pune district) have revealed several novel species and highlights the previously considered underestimated diversity of amphibians in Maharashtra. For example, endemic anuran genus *Xanthophryne*; species *X. tigerina*, *Indirana chiravasi*, *Pseudophilautus amboli*, *Micrixalus uttaraghati*, *Fejervarya cepfi*, *Gegeneophis danieli*, *Raorchestes ghatei*, *Indotyphlus maharashtraensis*, *Nyctibatrachus danieli*, *Indosylvirana caesari*, *Sphaerotherca pashchima* are

described in the last decade. Several researchers have worked on breeding behaviors, and found such as a new type of amplexus i.e. "Dorsal Straddle" with rare female call descript (Willaert et al. 2016; Gramapurohit et al. 2011) in endemic anuran *Nyctibatrachus humayuni*; reproductive biology of the endemic toad *Xanthophryne* (Gaitonde et al. 2016).

In the sense of tradition and religious duties, locals respect and protect their deity and their sacred groves (SGs). These groves are remnants of native forest covers so they contain high diversity and richness of species (Gadgil and Vartak 1976). The surrounding landscape influences the biodiversity thriving within the groves and any further disturbance may adversely affect the dwelling populations of endemic species and this may lead to local or permanent extinction of species in that area (Bhagwat et al., 2005). Sacred groves providing variety of habitats to locally restricted wild species as refuge so their protection should be on top priority (Ormsby and Bhagwat 2010).

The point endemism in amphibians has been seen on plateaus (PLs) in northern Western Ghats. It has been observed that plateaus acts as "islands" of biodiversity and

Xanthophryne tigerina is a critically endangered species.
Photo Credit: Abhijit Das



their local biological diversity is very unique than species found in plains. For example restricted distribution of *Xanthophryne tigerina* (Amboli plateaus), *X. koynaensis* and *Indotyphlus maharashtraensis* (Koyna plateaus) indicate extraordinary endemism for flora & fauna (Watve et al., 2013) in Northern Western Ghats. Presence of niches and micro habitats within forest patches and lateritic plateaus signify high amphibian conservation significance of these areas and they must be protected. This study adds more evidence to support this hypothesis.

Methods

Standard amphibian survey protocols were followed during the entire course of study. Stratified diurnal and nocturnal field surveys were conducted in mornings and evenings for two days per site for the inventory of endemic amphibians in the respective site using opportunistic sampling in the monsoon season from June to November in 2014. Survey time was approx. 6.00am-9.00am and 7.00pm- 10.00pm. Various habitat scanning methods were used such as actual sightings, presence of egg clutch, male anurans conspicuous advertisement calls, frogs' foam nest using search lights and head lamps at night. Previously published literature was reviewed to known the distribution and habitat of species. Variety of micro habitats such as leaf litter, rocky crevices, mud and small breeding pools for aquatic habitats, tree canopy for arboreal species were scanned. Digging the soil for fossorial animals such as *Ichthyophis* and *Indotyphlus*, upturning stones, scanning leaf litters; road kill sightings were also observed (Kamei et. al., 2009). Field protocols were followed such as using protective gum boots or shoes by field workers, head lamps and search lights were used for nocturnal surveys. First aid kit was always carried on the field (Gururaja and Ramachandra 2012). GPS readings of study sites were taken using GARMIN GPS 60. A final map using Arc GIS and Google Earth Pro was prepared.

Result

From our study we recorded seven endemic species which belong to seven families. Out of these seven Western Ghats endemics, six species *Fejervarya cepfi*, *Indirana leithii*, *Nyctibatrachus humayuni*, *Raorchestes ghatei*, *Indosylvirana caesari* and *Indotyphlus maharashtraensis* are endemic to Northern Western Ghats(*) i.e. they show restricted distribution with respect to north of Goa gap.

Figure 1: Map of Study sites and endemic species distribution



Table 1: Endemic species reported in this study:

Sgs					
S. no.	Species Common Name	Scientific Name	Family	Occurrence (as per IUCN	Conservation Status (IUCN)
1	CEPF Burrowing Frog	<i>Fejervarya cepfi</i>	Dicroglossidae	N/A	Not evaluated
2	*Matherana Leaping Frog	<i>Indirana leithii</i>	Ranixalidae	Common	Vulnerable
3	*Bombay Night Frog	<i>Nyctibatrachus humayuni</i>	Nyctibatrachidae	Common	Vulnerable
4	*Ghate's Bush Frog	<i>Raorchestes ghatei</i>	Rhacophoridae	Common	Data Deficient
PLs					
5	*Maharashtra Golden Frog	<i>Indosylvirana caesari</i>	Ranidae	Common	Data deficient
6	*Humbarli Caecilian	<i>Indotyphlus maharashtraensis</i>	Indotyphlidae	Uncommon	Data deficient
7	Marbled Ramanella Frog	<i>Uperodon mormoratus</i>	Microhylidae	Common	Endangered

Note: *Species endemic to Norther Western Ghats

Raorchestes ghatei was the most commonly encountered endemic species during our study and was sighted and heard in most of the sites in the shrubs and small trees in the sacred forest as well as on the shrub cover on high altitude plateaus. Newly described *Fejervarya cepfi* was uncommon and was found only in two sites, in a sacred grove and a plateau each. *Indirana leithii* was also fairly sighted in many of the sites under leaf litter, rocky outcrops and on the adjacent roads to sites. *Indosylvirana caesari* was seen on the rocks in the downside streams emerging from

sloppy edges of the Vankasawadi plateaus. *Indotyphlus maharashtraensis* was caught after digging the moist soil near the herbaceous plantation of *Strobilanthes* sp., *Eriocaulon* sp. and *Utricularia* on Vankasawadi Plateau.

Three species were threatened on IUCN redlist. *Indirana leithii* and *Nyctibatrachus humayuni* are Vulnerable; and *Uperodon mormoratus* is Endangered in IUCN redlist. The endemic species recorded in this study is approx. 35% of the total endemics of Northern western Ghats in

Hydrophylax bahuvistara
Photo Credit: Abhijit Das



Table 2: Site details and codes

S.no.	Surveyed Sites	Site Code	District	Latitude	Longitude	Elevation (approx. meters)
1	Bapuchibuva Sacred Grove	SG1	Pune	18°31'14.04"N	73°23'36.43"E	712
2	Ghataidevi Sacred Grove	SG2	Satara	17°41'44.74"N	73°50'11.41"E	1150
3	Kondethar Sacred Grove	SG3	Raigad	18°23'54.80"N	73°23'47.82"E	539
4	Vadavdhar Sacred Grove	SG4	Pune	18°33'39.90"N	73°29'9.95"E	757
5	Valne Sacred Grove	SG5	Pune	18°32'58.13"N	73°30'11.69"E	735
6	Yavteshwar Sacred Grove	SG6	Satara	17°41'27.79"N	73°57'9.47"E	1041
7	Chalakewadi Plateau	PL1	Satara	17°33'0.52"N	73°50'17.66"E	1143
8	Durgawadi Plateau	PL2	Pune	19°13'10.60"N	73°38'59.38"E	1157
9	Hatvij Plateau	PL3	Pune	19°12'33.62"N	73°38'45.32"E	1105
10	Vankasawadi Plateau	PL4	Satara	17°27'1.57"N	73°50'0.93"E	1085
11	Warsubai Plateau	PL5	Pune	19°11'22.88"N	73°42'48.49"E	1130
12	Yavteshwar Plateau	PL6	Satara	17°42'3.91"N	73°55'19.24"E	1025

Table 3: Presence chart of endemic species in study sites

S. no.	Species	SG 1	SG 2	SG 3	SG 4	SG 5	SG 6	PL 1	PL 2	PL 3	PL 4	PL 5	PL 6
A	<i>Fejervarya cepfi</i>					Y					Y		
B	<i>Indirana leithii</i>	Y			Y	Y	Y		Y		Y	Y	
C	<i>Nyctibatrachus humayuni</i>	Y	Y										Y
D	<i>Raorchestes ghatei</i>	Y	Y		Y	Y	Y			Y	Y	Y	
E	<i>Indosylvirana caesari</i>										Y		
F	<i>Indotyphlus maharashtraensis</i>										Y		
G	<i>Uperodon mormoratus</i>										Y		

Maharashtra. More detailed assessments are required in such sacred groves and plateaus as they will yield more endemic species thriving in legally unprotected areas and their conservation measures are needed to be addressed urgently since landuse and land cover patterns are changing rapidly in these landscapes due to urbanization and developmental activities.

Species account

1) Matheran Leaping Frog *Indirana leithii* (Boulenger, 1888)

Diagnostic Characters: Medium-sized frog with snout vent length (SVL) 34mm; head longer than wide and snout lengthier than horizontal diameter of eye; snout rounded; pupil horizontal; tympanum

distinct, more than¾ of eye; canthus rostralis indistinct in cross section; flank granular with warts; has truncated and Y shaped discs. Femoral glands are present in males. First finger shorter than second, webbing extensive. Semi-terrestrial tadpoles.

Distribution: Restricted to northern Western Ghats from Ahwa Dang in Gujrat to Gaganbawda in Maharashtra, records outside Western Ghats needs confirmation (Dahanukar et al. 2016). Recorded and found common in Bhimashankar, Matheran and Mahabaleshwar.

Local Observations: *I. leithii* was found and seen commonly in most of the sites. Individuals were seen in the moist leaf litter and wet rocks crevices in forest patches of sacred grove and plateaus in Mulshi, Junnar (Pune) and Patan Koyna (Satara). We



Figure : Matheran Leaping Frog
(*Indirana leithii*)

observed few dead (road killed) individuals on tar road adjacent to Vadavthar sacred groves after heavy showers in the night. On an occasion we observed *I. leithii* individuals moving out from the plastic and temple waste in Valne sacred grove (Pune district). They were more abundant in sacred grove forest areas than plateau grasslands (see table 3).

2) CEPF Burrowing Frog *Fejervarya cepfi* (Garg and Biju, 2017)

Diagnostic Characters

It's a medium sized frog (SVL 33 mm) with stout body. Snout subovoid in dorsal view

and obtuse in lateral view. Presence of rectal gland at labial region of the mouth; eye length shorter than snout length; tympanum diameter nearly half of eye length; thigh length shorter than shank length; prominent shovel-shaped inner metatarsal tubercle prominent and small outer metatarsal tubercle; webbing between toes small.

Distribution

Known to occur in Western Ghats in Maharashtra. Amboli (Sindhudurg district) is the type locality. Also shows confirmed distribution record from Phansad Wildlife Sanctuary (Raigad district) and Koyna Wildlife Sanctuary in Satara district (Garg and Biju, 2017).

Figure : CEPF Burrowing Frog
(*Fejervarya cepfi*)



Local Observations

We recorded this species from Valane sacred grove (Pune) and Vankasawadi Plateau (Satara) in our study (see table 3). It was relatively less abundant as compared to its sympatric *F. syhadrensis*. On both occasion we found them on tar roads at night after light showers.

3) Bombay Night Frog *Nyctibatrachus humayuni* (Bhaduri and Kripalani, 1955)

Diagnostic Characters

N. humayuni is a medium sized frog (male 47mm, female 52mm), snout is rounded; tympanum is not visible; grey, brown to brick red and wrinkled dorsal skin with dorsolateral folds and glandular projections; well-developed ridge extending from the lip over the tip of the snout to between the nostrils, at which point it bifurcates, producing an inverted 'Y' and webbing is modest (Biju et. al. 2011).

Distribution

N. humayuni is known to occur and widely spread in the semi evergreen forests in Koyna Kaas, Mahabaleshwar and Bamnoli in Satara district; Khandala, Mulshi in Pune district; and Matheran in Raigad district.

Local Observations

We found this species from two sacred forest and two plateaus edges which had somewhat good overhanging vegetation with canopy especially in both sacred groves (see table 3). All the recorded sites had perennial rocky streams. It has been observed in Ghatai Devi sacred grove where the stream water was diverted for construction purpose to expand the temple premises may degrade the forest patch and subsequently impact the habitat of *N. humayuni*. It is the only anuran species in the world known to use a newly discovered mating position known as "dorsal straddle" and rare female calls of *N. humayuni* has been reported (Willaert et al. 2016). It is an endemic frog and Vulnerable on IUCN redlist. Its conservation measures are recommended to stop infrastructural



Figure : Bombay Night Frog
(*Nyctibatrachus humayuni*)

activities in the sacred groves and surrounding forest.

4) Ghate's Shrub Frog *Raorchestes ghatei* (Padhye, Sayyed, Jadhav, and Dahanukar, 2013)

Diagnostic characters

It is a shrub frog with size (25mm); snout

slightly projecting beyond mouth; tympanum small 1/3 of eye. Tongue deprived of papilla; nuptial pad is absent; a bony tubercle on humerus in males is present as sexual dimorphism character. Skin is granulated on dorsum; lateral groin is marbled with white blotches on brown; fingertip dilation has discs.

Distribution

It is a common and widely distributed species inhabit semi-evergreen forests and scrub patches in the Western Ghats of Maharashtra.

Local Observations

The only shrub frog species recorded in our study. Common in most of the sites. Males were heard calling from shrubs and undergrowth at height of 1-5 meters above ground at night. Some individuals (mostly females) were seen under rocks in day.

Figure : Ghat's Shrub Frog
(*Raorchestes ghatei*)



5) Maharashtra's Golden-backed Frog *Indosylvirana caesari* (Biju, Mahony, Wijayathilaka, Seneviranthne, and Meegaskumbura, 2014)

Diagnostic Characters

I. caesari is a medium sized frog with 50mm SVL). Head is small, extended; horizontal from dorsum; snout is pointed in dorsal and ventral and rounded in lateral view. canthus rostralis cross section is rounded; nostril oval; tympanum slightly smaller than of eye; Forelimbs shorter than the length of hands; fingers are long; pointed discs; moderate webbing present; Skin near snout and eyes shagreened; dorsum granular; dorsolateral fold present; rictal gland is distinct at corner of mouth; throat smooth; ventral belly shagreened.

Distribution

It's endemic to Western Ghats of Maharashtra. Recorded from Humbarli in Koyna, Bamnoli, Kaas, Mahabaleshwar (Satara district) and Amboli (Sindhudurg district) (Biju et al. 2014). It has been seen in streams of north Goa also.

Local Observations

During our study, this species was recorded only from Vankasawadi Plateau area in Patan (Satara district) in a sloppy downside hill stream at the edge of plateau. Three individuals (males) were seen on the lateritic rocks in the stream near monsoon ephemeral grown herbaceous vegetation of *Eriocaulon* sp. and *Utricularia* sp. on the upper side and rocky stream in the downside of the hill. This area is under severe anthropogenic pressure due to windmill farms and construction sites.



Figure : Maharashtra's Golden-backed Frog (*Indosylvirana caesari*)

6) Marbled Ramanella
***Uperodon marmorata* (Rao, 1937)**

Size approx. 35 mm. Blunt snouts with V shaped markings on snout. Warty dorsum. Fingers with wide triangular dilations. Less webbing in feet. Occur in north Western Ghats montane rain forests above 1,000 m and north Western Ghats moist deciduous forests below and can be seen on the forest floor, tree bases, holes and termite mounds. Nothing is known about its breeding

behavior, probably its eggs metamorphose into adults through larval development like other microhylids. Habitat loss is caused due to change in land use pattern for agriculture, plantation, tourism and development are amongst major threats to this anuran. In our surveys to Vankaswadi plateau, we have observed an individual in a small forest patch near road. Habitat fragmentation further drives this species towards extinction. It's an Endangered species on IUCN redlist and endemic to Western Ghats.

Figure : Marbled Ramanella (*Uperodon marmorata*)



7) Humbarli Caecilian
Indotyphlus maharashtraensis
(Giri, Wilkinson, and Gower,
2004)

Diagnostic Characters

Secondary annular grooves present; it has a depressed preanal strip anterior to disc surrounding vent. Small mid-ventral longitudinal incision in front of vent; V shaped scar on the posterior part of dorsal surface of head; Body shape cylindrical and dorsally compressed. Snout tip bluntly rounded; Eyes visible through skin;

Tentacles are short and globular and pointed at tip. Tentacles apertures are horseshoe shaped.

Distribution

Known to occur only from its type locality in Koyna (Satara district).

Local Observations

It was seen in the lateritic moist thin soil cover after digging on the high altitude plateau top of Vankasawadi in Patan post monsoon in September 2014. The surrounding habitat had sparsely grown *pleocaulus* sps., *Eriocaulon* sps. and *Utricularia* sps. herbaceous vegetation.



Figure : Humbarli Caecilian
(*Indotyphlus maharashtraensis*)

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Larval taxonomy of Indian Anurans

Abstract

Keys for tadpoles of 38 species of anurans from India belonging to various genera collected from various aquatic systems are herein described. For the current study, we used a set of external, buccopharyngeal and internal anatomical characters to build a robust taxonomic key system that would facilitate in proper taxonomic identification of anuran larvae from the Indian subcontinent.

Introduction

Most amphibians especially anurans, have a larval stage that is morphologically different from their adult forms. In general, anuran larvae live in different ecological setups than the adult, have different physiological and dietary requirements, and have different predators while having the same set of selection pressure as of the adults. During this larval phase, the tiny larva (tadpole) develops from an aquatic gill-breathing organism with a tail to a lung breathing four-legged metamorph. In addition, many species have highly specialized larval morphologies, with various morphological characters essential for their survival that are modified or lost upon metamorphosis. Traditionally, anuran taxonomy was mainly focused on variation and characteristics of adults with a few studies dealing with larval morphology. The larval stages of anurans are termed as "tadpoles" which was derived from medieval english term "taddepol" (tadde- toad and pol-head). Physically the tadpoles resemble fishes with a large body and an elongated caudal tail. Research on tadpole morphology has grown rapidly in recent years, but many larval anurans remain unknown. Unfortunately, difficulties in tadpole identification had been a major obstacle in

anuran systematics and conservation programs.

Tadpole work in India

Indian subcontinent is home to more than 350 species of anurans of which of which ~ 60 species have direct development and the rest have free-living larval forms (tadpoles). Of these species having free-living tadpoles, larval (tadpole) identities of ~100 species

Key words:

Tadpole; External morphology; Buccopharyngeal; Chondrocranium; Morphological key.

Stream living tadpoles show special characters for adaptation in torrent habitat.
Photo Credit: Abhijit Das

Prudhvi Raj¹, Karthikeyan Vasudevan², Gunanidhi Sahu³ & Sushil K. Dutta³

¹ Wildlife Institute of India, Chandrabani, Dehradun: 248001, Uttarakhand, India

² Department of Zoology, Utkal university, Vani Vihar, Bhubaneswar: 751004, Odhisa, India.

³ Laboratory for Conservation of endangered species (LaCONES), Centre for Cellular & Molecular biology, CSIR-CCMB, Hyderabad 500007, India.

Corresponding author: e-mail: prudhviraj999@gmail.com



are known. In comparison to work on adult anurans from India, little detailed systematic work has been carried on the larval stages. By early 20th century, biologists started describing larval forms for many anurans from the Indian subcontinent and provided characters for their identification. Annandale and Rao during this period has taken the lead and made extensive morphological descriptions for larvae mostly on peninsular Indian anurans and a few from the Himalayas. Work by Annandale (1905; 1906; 1908; 1912; 1913; 1917; 1918; 1919), Annandale & Hora (1922), Annandale & Rao (1917; 1918) and Rao (1914; 1915; 1918; 1922; 1937; 1938) are the most comprehensive that has appeared so far. Later during the middle of last century larval descriptions for a few species were made by Bhaduri (1935; 1944a; 1944b), Bhaduri & Daniel (1956), Bhaduri & Kripalani (1954), Kripalani (1953a; 1953b). This was followed by Agarwal & Niazi (1977; 1980), Bhatia (1969), Bordoloi et al. (2001), Chanda & Talukdar (1973), Chari (1962), Daniel (1962; 1963a; 1963b; 1975), Das (1996; 1998), Dubious (1974; 1976), Dutta (1997), Dutta et al. (1993; 1994; 2001; 2004), Dutta & Mohanty-Hejmadi (1976; 1984), Hiragond et al. (2001), Inger (1985), Khan (1965; 1969; 1982a; 1997; 2002; 2003a; 2003b; 2003c; 2004), Khan et al. (1979), Khare & Sahu (1984), Kuramoto & Joshy (2002), Leong (2002; 2004; 2005), Mallick et al. (1979), Mehta (1983), Mohanty et al. (1996), Mohanty-Hejmadi & Dutta (1979),

Pillai (1978), Sahu (1994), Sahu & Khare (1980; 1983), Sekar (1990a; 1990b; 1992) in the later part of last century and early this century. However, many of these works on tadpole external morphology were inadequately characterized. On the anatomical studies, pioneering work has been carried by Ramaswami (1932; 1933; 1934; 1936; 1938; 1940; 1943; 1944) on musculature and chondrocranial morphology of many larval anurans from India which still remains to be the only complete comparative anatomical studies on tadpoles of India till date. This was followed with work by Chaco (1965) and Khan (2003d); however, after this no subsequent work was carried on material from India. Work on buccopharyngeal anatomy of tadpoles of a few anuran species from India was taken up by Das (1994), Grosjean (2003; 2004) and Hora (1922). Buccopharyngeal studies of few species with extra-limital distribution were taken up by Khan (1996a; 1996b; 2000), Khan & Malik (1987), Khan & Mufti (1994; 1995). Few keys to anuran larval faunas from India are available. However, subsequent to the general larval keys for few anurans from India and Pakistan by Annandale (1918), Annandale & Rao (1917; 1918), Khan (1982) and Sahu (1994) there have been no attempts to provide comprehensive keys to Indian tadpoles. In the past few decades, there had been major systematic rearrangements of many anuran species and several new genera were described

Metamorph of *Hyla annectans*, Nagaland.
Photo Credit: Abhijit Das



with considerable amount of new information. Tadpoles of species restricted to known hotspots, such as the Western Ghats and the Eastern Himalayas in India, remain poorly known, especially since spectacular discoveries have been made in the recent past from these regions.

Taxonomy of anuran larvae

Tadpoles of most species tend to have similar body forms and which makes them notoriously difficult to identify for a layman. Morphologically tadpoles of most species show homoplasy and distantly related species have very similar appearing tadpoles. There are few keys available for identifying tadpoles to species. Tadpoles are generally identified using a set of characters. The most important character is the denticle row formulae (LTRF- Labial Teeth Tow Formula) (Fig 1). However, going by LTRF alone would lead to ambiguity. One needs to identify them using a set oral characters and a set of external morphological characters. In many groups, larval morphology is conserved and tadpoles of many species of the same genus tend to look alike. Tadpoles due to various ecological factors have different anatomies and show considerable variation during the ontogeny in terms of their morphology. However, they do have certain morphological traits that can be used to distinguish among species. Recent advances in molecular studies had made identification of tadpoles easier and one can easily ascertain the taxonomic identity of the tadpole.

Methods

External morphology

There is a lot of confusion on the usage of terminology on anuran tadpole morphology. For external morphological and oral disc terminology, it is advised to follow Altig & Johnston (1989), and Altig & McDiarmid (1999). In recent years, Altig (2007) and Haas (2011) summarized much of the known information. For a layman, taxonomic identity of anuran larvae can be done using some simple external (Fig 2) and oral morphological keys. Externally tadpoles can be differentiated based on the position of the oral disc, position of the eye, opening of

the vent tube, shape of the tail, etc. With in oral disc, one needs to look into the shape of the jaw sheath, pigmentation of the jaw sheath, nature of the oral disc, distribution of marginal papillae, pigmentation on the jaw sheaths, presence/absence of denticle rows, indentations of the oral disc, etc. Tadpoles of anurans during their ontogeny are referred by various developmental stages usually defined by various morphological attributes. One of the most widely used staging tables was the one developed by Gosner (1960) primarily based on hind limb development. The developmental stages of anurans can be broadly grouped into two phases: (i) the initial growth phase, which is characterized by an aquatic free-swimming tadpole (Gosner stage 25-40) and; (ii) the metamorphosis phase (Gosner stage 40-46), during which the tadpole loses the free-swimming neonatal characters and starts to resemble the adult. Morphometric measurements of various morphological variables are of great taxonomic value and can be useful in taxonomic identification of tadpoles.

Buccopharyngeal study

The internal buccal and the pharyngeal anatomy of tadpoles vary across species. The internal surface of buccopharyngeal region is dotted with a number of chemoreceptory pustules and papillae (Fig. 3). The number and nature of these structures is species specific and can be of great taxonomic importance. These internal structures can be studied either by using photographs taken by Scanning Electron Microscope or by simple staining of internal structures using Methylene Blue and observing under stereo zoom microscope (SEM). Careful dissection of the tadpole by making incisions through the lateral corners of the jaw sheath would divide the buccal region into dorsal (buccal roof) and ventral (ventral floor) regions. For standard terminology and methods for studying buccopharyngeal structures of tadpoles, one can refer to published literature by Wassersug (1976, 1980) and Inger (1985).

Anatomical characters

Anatomical characters are more conserved

and can be useful in ascertaining phylogenetic relationships. Chondrocranium along with the ceratohyal forms the major structural composition of the tadpole anatomy (fig. 4). Despite this, so far there few good studies on larval anatomy and little is known about the function of the chondrocranium and the concerned musculature or how developmental factors contribute to inter-specific morphological variation. One of the easiest ways of studying larval anatomy is through staining. Anuran larval anatomy during the tadpole stage constitutes only cartilage and gets replaced with bone during metamorphosis. Alcian blue is used to stain cartilage and Alzarin red is used for staining bone.



A typical stream habitat showing various microhabitats.

Results

Larval keys

Based on this morphological characterization, taxonomic key for tadpoles of 38 anuran species are being produced.....		1
1 A.	Without nares; without jaw sheath, spiracle ventral;.....	2
B.	With nares; with jaw sheaths, spiracle sinistral;.....	4
2 A.	Oral disc with a fold on the lower labium with no denticle and papillae; one medial vent and a medial spiracle; large lateral eyes with a transparent body	3
B.	Oral disc with a fold on the lower labium with no denticle and papillae; dorsal side speckled with reddish brown melanophores; small lateral eyes with a translucent body diffused with many melanophores.	<i>Uperodon systoma.</i>
3 A.	Dorsum with a diamond marking due to congregation at the centre of the abdomen; Caudal fin with melanophores spread uniformly	<i>Microhyla berdmorei.</i>
B.	Dorsum with uniformly spread tiny melanophores; Caudal fin with melanophores concentrated at the distal end of the fin	<i>Microhyla ornata.</i>
4 A.	Oral disc with multi lobed oral disc and without labial tooth rows, dorsal fin origin posterior to body tail junction	5
B.	Oral disc with emarginated or non-emarginated oral disc with labial tooth rows	6
5 A.	Small tadpoles, with body length less than 11 mm (stage 35-40), tadpoles with pale body and sparsely dotted with few brown melanophores	<i>Nyctibatrachus cf. minor.</i>
B.	Large tadpoles, with body length more than 11 mm (stage 35-40), tadpoles with dirty brown body colouration	<i>Nyctibatrachus cf. major.</i>
6 A.	Eyes lateral/ near lateral; mouth anteroventral; origin of the ventral fin anterior to body tail junction	7
B.	Eyes dorsal/ dorsolateral; mouth ventral/ anetero-ventral; origin of the tail at body tail junction/posterior to body tail junction	11

-
- 7 A. Eyes near lateral; vent tube medial; both fins of equal height; marginal papillae on the oral disc distributed with wide dorsal gap; single row of marginal papillae; jaw sheath was broadly rounded with a long lateral process (trapezoidal) *Hyla annectans*.
- B. Eyes lateral; vent tube dextral; ventral fin taller; marginal papillae on the oral disc distributed with wide dorsal gap and narrow ventral gap; marginal papillae were single row on the anterior labium and double row on the posterior labium; jaw sheath arch shaped 8 A. Opening of the nostrils circular/oval with the rim elevated or depressed; pre narial arena with an arched medial ridge and many secondary lateral papillae or a simple arched medial ridge; Infralabial papillae pointed with rugose margin; lingual papillae smooth 9
- B. Opening of the nostrils reniform with rim elevated; pre narial arena with arched medial ridge and many secondary lateral papillae; Infralabial papillae dilated; lingual papillae postulated 10
-
- 9 A. Nostril opening depressed; pre narial arena of the buccal roof was simple arched medial ridge; four lingual papillae; A5 (4)/P3 (1) *Polypedates cf. pseudocruciger*.
- B. Nostril opening elevated; pre narial arena of the buccal roof was arched medial ridge with many secondary lateral papillae; two lingual papillae; A4 (3)/P3 (1) *Polypedates maculatus*.
-
- 10 A. two lingual papillae; A5 (4) (1)/P3 (1) *Polypedates cf. himalayanus*.
- B. Four lingual papillae; A4 (3)/P3 *Polypedates teraiensis*.
-
- 11 A. Eyes dorsal placed back on the dorsum; oral disc large and ventral; tail muscle wide in dorsal view; origin of the tail posterior to body tail junction 12
- B. Eyes dorsolateral placed near the anterior end of the body on the dorsum; oral disc small to moderate and positioned either ventral/anterioventral; tail muscle wide/narrow in dorsal view; origin of the tail at body tail junction/ posterior to body tail junction 13
-
- 12 A. Absence of cutaneous granular glands on the body; absence of suction organ formed by gastrozymus muscle; vent tube medial; labial tooth row formula was A 2/P3 (1) *Nasikabatrachus sahyadrensis*.
- B. Presence of cutaneous granular glands on the body; presence of suction organ formed by gastrozymus muscle; vent tube dextral; labial tooth row formula was A8 (5)/P3 (1) *Amolops assamensis*.
-
- 13 A. Presence of cutaneous granular glands on the body 14
- B. Absence of cutaneous granular glands on the body 17
-
- 14 A. Large tadpole; presence of parotid gland, posteroventral gland and supracaudal gland 15
- B. Moderate sized tadpole; presence of parotid gland, posteroventral gland and absence of supracaudal gland; labial tooth row formula is A2 (1)/P3 (1) 16
-
- 15 A. presence of ocellus on the caudal musculature; labial tooth row formula is A6 (4)/P7 (1). *Clinotarsus alticola*.
- B. Absence of ocellus on the caudal musculature; labial tooth row formula is A8 (6)/P7 (1). *Clinotarsus curtipes*.
-
- 16 A. Nostrils located midway between the eye and snout; internal nares narrowly separated; Buccal Roof Arena (BRA) papillae were abundant; straw coloured body with few melanophores *Indosylvibana indica*.
- B. Nostrils located midway between the eye and snout; internal nares moderately separated; BRA papillae were fewer; Body straw coloured but with many melanophores *Indosylvibana indica*.

C.	Nostrils closer to eye; internal nares narrowly separated; BRA papillae were fewer; Body dirty yellow with numerous tiny brown melanophores	<i>Indosylvibana indica</i> .
17 A.	Vent tube opening medial	18
B.	Vent tube opening dextral	23
18 A.	Jaw sheath broadly rounded with long lateral process (trapezoidal); caudal musculature strong with low dorsal and ventral fin	19
B.	Jaw sheath with elongated thick middle portion and thin lateral processes; caudal musculature strong with low dorsal and ventral fin	22
19 A.	Centripetal wall of the spiracle totally fused to the body wall with distal end free; broadly rounded tail	20
B.	Centripetal wall of the spiracle partly formed; rounded tail	21
20 A.	Shape of the narial opening was oval/rounded; both caudal fins of equal height; jaw sheath keratinization moderate; median ridge bifid; BRA pustules few	<i>Duttaphrynus himalayanus</i> .
B.	Shape of the narial opening was reniform; Dorsal fin taller; jaw sheath keratinization weak; median ridge trifid; BRA pistules absent	<i>Bufo latastii</i> .
21 A.	Body colour black olive; ethmoid plate of the chondrocranium elongated; coupla 1(Basihyal) present; Gap below the hypobranchial plate was wide	<i>Duttaphrynus melanostictus</i> .
B.	Body colour black olive with golden speckles; ethmoid plate of the chondrocranium intermediate; coupla 1(Basihyal) absent; gap below the hypobranchial plate was intermediate	<i>Duttaphrynus cf. melanostictus</i> .
22 A.	Semi terrestrial tadpoles; large dorsolateral eyes; triangular body; oral disc positioned anterolateral; Spiracle with no centripetal wall and tube reduced/dumitive	<i>Indirana cf. beddomii</i> .
23 A.	Presence of external naso-lacrimel groove	24
B.	Absence of external naso-lacrimel groove	26
24 A.	Narial opening elevated; nostrils closer to the eye; oral disc with a wide dorsal gap and narrow ventral gap; Fewer BRA and Buccal Floor Arena (BFA) pustules; labial tooth row formula was A5 (4)/A3 (1)	<i>Rhacophorus maximus</i> .
B.	Nostrils placed midway; oral disc with wide dorsal gap; abundant BRA and numerous BFA pustules	25
25 A.	nostril opening shape oval/rounded; dorsal fin taller; median ridge triangular; labial tooth row formula was A6 (4)/A3 (1)	<i>Rhacophorus lateralis</i> .
B.	Nostril opening shape oblong; both fins are of equal height; median ridge triangular; labial tooth row formula was A7 (5)/3(1)	<i>Rhacophorus malabaricus</i> .
C.	Nostril opening shape oblong; dorsal fin taller; median ridge bifid; labial tooth row formula was A6 (4)/3(1)	<i>Rhacophorus pseudomalabaricus</i> .
26 A.	Oral disc located ventrally	27
B.	Oral disc located antero ventrally	29
27 A.	Shape of the nostril oval/round; both fins of equal height; marginal papillae distributed on the oral disc with narrow dorsal and ventral gaps; submarginal papillae present on both labia; shape of upper jaw sheath Inverted v-shaped with a medial protrusion; no pre narial medial ridge; labial tooth row formula is A5 (4)/P6 (6)	<i>Leptobrachium smithi</i> .
B.	Shape of the nostril opening reniform; marginal papillae distribution on the oral disc with a wide gap on the upper labium; submarginal papillae present at the lateral corners and lower labium of the oral disc; Jaw sheath keratinization strong; upper jaw sheath arch shaped	28

28. A.	Snout broadly rounded; widest part of the body middle of the abdomen; broadly rounded tail tip; simple arched pre medial ridge; labial tooth row formula was A6 (4)/P3 (1)	<i>Nanorana vicina</i> .
B.	Snout rounded; widest part of the body at the back of the abdomen; rounded tail tip; single papilla pre medial ridge; labial tooth row formula was 5(4)/3(1);	<i>Nanorana minica</i> .
29. A.	Opening of the nostrils depressed; marginal papillae were uniseriate on upper labium and biseriate on the lower labium; shape of the upper jaw sheath Inverted v-shaped with a medial protrusion	30
B.	Opening of the nostrils elevated	31
30. A.	Infra-labial papillae shape dilated	<i>Chiromantis dudwaensis</i> .
B.	Infra-labial papillae shape pointed	<i>Chiromantis simus</i> .
31. A.	Infra-labial papillae shape dilated	32
B.	Infra-labial papillae shape bifurcate/ compressed	33
32. A.	nostril located closer to the eye; tail tip pointed; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization weak; upper jaw sheath arch shaped with medial protrusion; internal nares widely separated; BRA pustules few; two lingual papillae; labial tooth row formula is A2 (1)/P3	<i>Fejervarya cf. keralensis</i> .
B.	Nostril located midway between eye and snout; tail tip pointed; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization weak; upper jaw sheath broadly rounded with a short lateral process and a medial protrusion; internal nares narrowly separated; BRA pustules abundant; two lingual papillae; labial tooth row formula is A2 (1)/P3 (1).	<i>Fejervarya cf. orissaensis</i> .
C.	Nostril located closer to the eye; tail tip rounded; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization moderate; upper jaw sheath arch shaped with medial protrusion; internal nares moderately separated; BRA pustules abundant; two lingual papillae; labial tooth row formula is A3 (2)/P3	<i>Fejervarya cf. pierrie</i> .
D.	Nostril located closer to the eye; tail tip rounded; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization moderate; upper jaw sheath arch shaped with medial protrusion; internal nares moderately separated; BRA pustules abundant; four lingual papillae; labial tooth row formula is A2 (1)/P3	<i>Fejervarya cf. limnocharis</i> .
E.	Nostril located midway to the eye; tail tip pointed; submarginal papillae absent Jaw sheath keratinization moderate; upper jaw sheath arch shaped with medial protrusion; internal nares moderately separated; BRA pustules few; four lingual papillae; labial tooth row formula is A2 (1)/P3 (1)	<i>Fejervarya cf. nilagarica</i> .
33. A.	Widest part of the body was at the back of the abdomen; submarginal papillae absent; jaw sheath keratinization strong; Jaw sheath arch shaped with medial protrusion; labial tooth row formula is A1/P2	<i>Euphlyctis cyanophlyctis</i> .
B.	Widest part of the body was at the middle of the abdomen; submarginal papillae at the lateral corners of the oral disc; jaw sheath keratinization weak; Jaw sheath arch shaped with medial protrusion; labial tooth row formula is A2 (1)/P3	<i>Sphaerothera breviceps</i>



Tadpole of *Indirana* sp. are semi-aquatic.

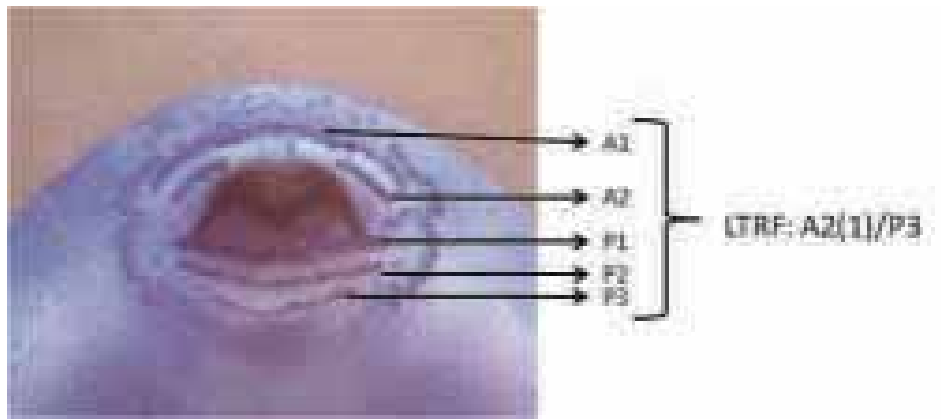


Figure 1: Labial Tooth Row Formula (LTRF) of *Fejervarya* cf. *nilagirica*

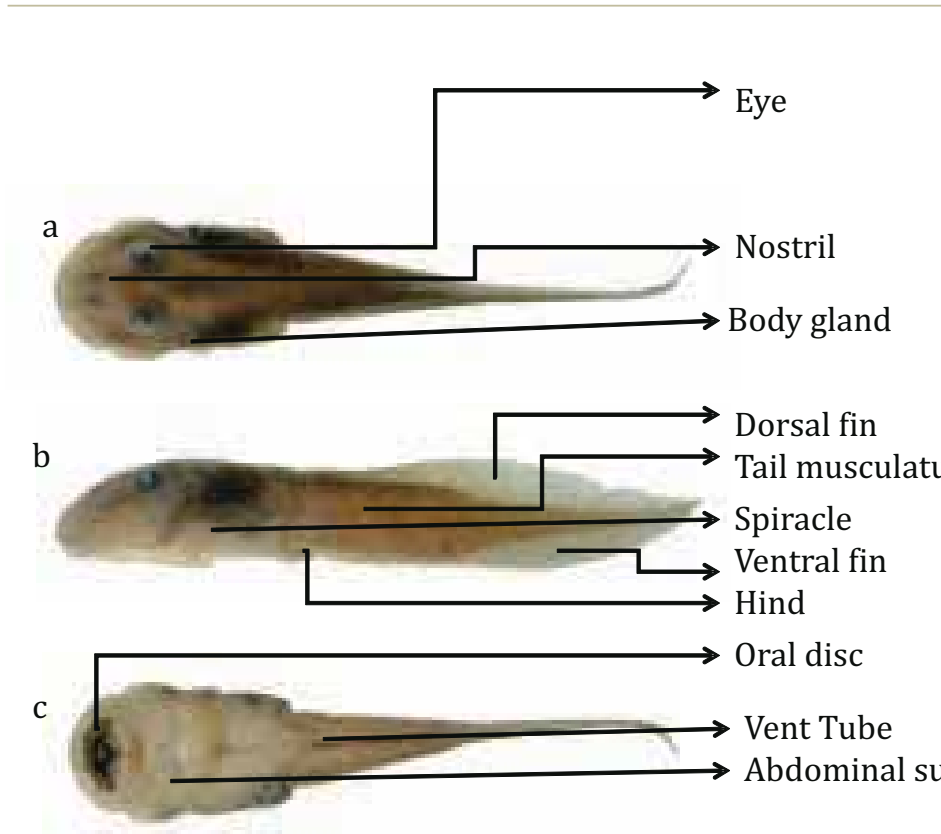
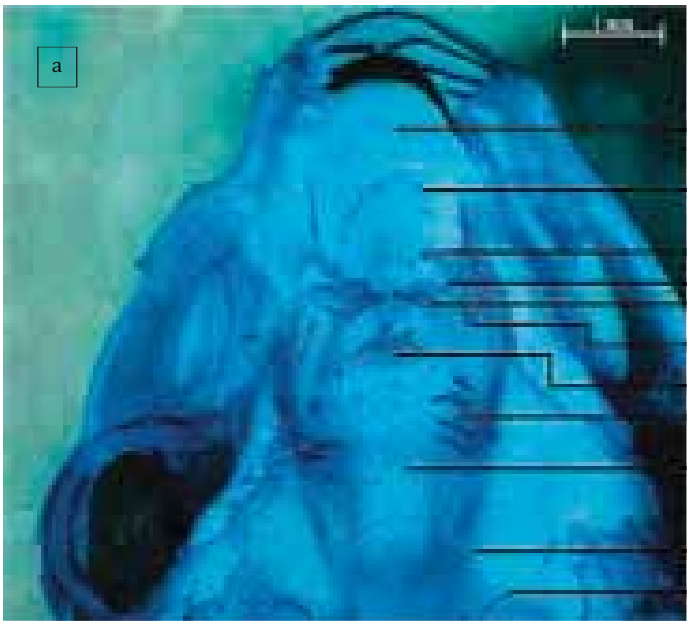
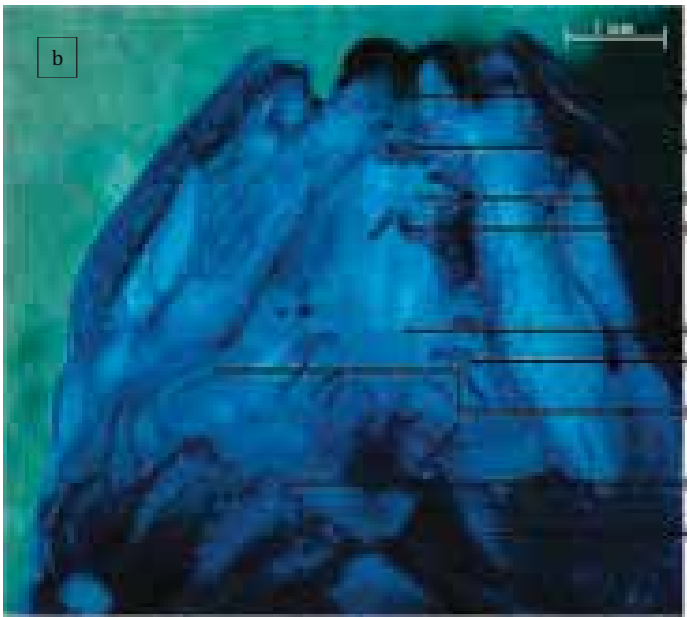


Figure 2: *Amolops assamensis*, Gosner stage 37. External morphology (a) Dorsal view; (b) Lateral view; (c) Ventral view.



- Pre-narial arena
- Pre-narial ridge
- Anterior narial wall papilla
- Posterior narial wall
- Posterior narial papilla
- Lateral ridge papilla
- Median ridge papilla
- Buccal roof arena papilla
- Buccal roof arena pustules
- Glandular zone
- Dorsal velum



- Pre-lingual arena
- Pre-lingual papillae
- Tongue anlage
- Lingual papillae
- Buccal floor arena
- Buccal floor arena papillae
- Buccal pockets
- Ventral velum
- Gill clefts

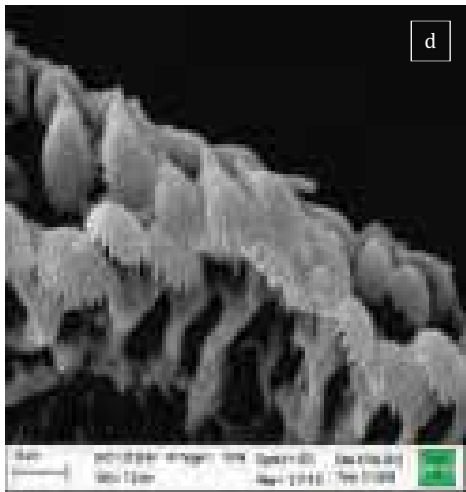


Figure 3: *Polypedates cf. occidentalis*, stage 37. Buccopharyngeal and denticles (a) Buccal roof, Methylene Blue stained photograph, ventral view, scale = 1 mm; (b) Buccal floor, Methylene Blue stained photograph, ventral view, scale = 1 mm; (c) Individual denticles, scale = .1 mm; (d) Jaw sheath serrations = .1 mm.

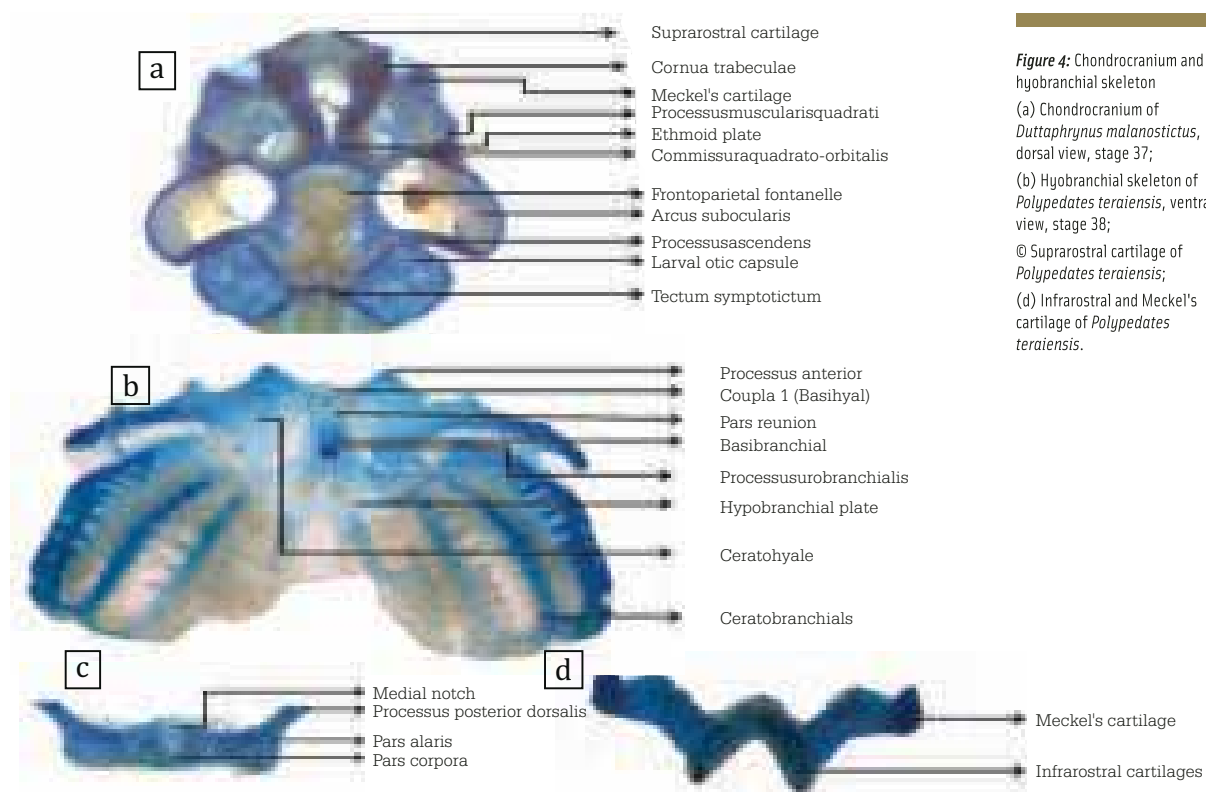


Figure 4: Chondrocranium and hyobranchial skeleton
(a) Chondrocranium of *Duttaphrynus melanostictus*, dorsal view, stage 37;
(b) Hyobranchial skeleton of *Polypedates teraiensis*, ventral view, stage 38;
(c) Suprarostril cartilage of *Polypedates teraiensis*;
(d) Infracrostral and Meckel's cartilage of *Polypedates teraiensis*.

Glossary

Anterior: front of the body

Basihyal cartilage (Coupla 1): cartilage that chondrifies between the anterior tips of the ceratohyal cartilage

Bucco- Pharyngeal: the region starting from the buccal to the pharynx or nasal passage, or (b) the most ventral element of a gill arch

BRA: Buccal roof arena

Branchial arches: The successive bulges in the lateral walls of the pharynx found between successive branchial groove and branchial pouches or both

Choanae: Internal narial opening

Chondrocranium: The anterior part of the axial skeleton that encases the special sense organs and contributes to the skeletal elements encasing the brain

Caudal: Toward the tail end, inferior

Dextral: Right side

Ethmoid plate: bone that separates the nasal cavity from the brain

Fontanelles: Temporary openings between bony plates of the skull, formed during the formation and fusion of the cranial bones

Infracrostral cartilage: Cartilage which acts as a precursor to the lower jaw

Jaw sheath: A chondrofed structure present inside the oral disc used for engulfing food

Lateral: towards the sides

Melanophore: Pigment cell that contains melanin

Neurocranium: Portion of the cranium that houses the brain

Papillae: Small, nipple like projections

Pistules: Tiny projections

Posterior: Nearer the back of the body, dorsal

Spiracle: A tube through which water is released from the body

Sinistral: Left side

Suprarostril cartilage: Cartilage found attached at the anterior end of the chondrocranium and supports the jawsheath

Vent tube: A tube at the posterior end of the body for discarding excreta

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Survey and monitoring of salamander populations in the Darjeeling Himalaya of northern West Bengal

Abstract

The Himalayan Salamander is the only species of salamander found in the Darjeeling Himalayas of northern West Bengal and is protected under Schedule II of the Indian Wildlife (Protection) Act, 1972. Even the distribution of the species is not clearly known due to lack of proper surveys. We conducted a status survey of this species during the monsoon seasons of 2005-2007 and located it in 21 small and medium-sized wetlands in the Darjeeling hills and tried to estimate its breeding population in them. We also tried to determine the possible causes of its rapid decline to help in formulating a conservation action plan for the species in future.

Introduction

The Himalayan Salamander *Tylototriton himalayanus* (Khatiwada et al. 2015) is the only species of salamander (Amphibia: Urodela: Pleurodelidae) that is found in the Darjeeling Himalayas of northern West Bengal. It is a unique and rare tailed amphibian that is protected under Schedule II part II of the Indian Wildlife (Protection) Act, 1972. It is a bioindicator species of the lentic habitats in the eastern Himalayas. It occurs between the altitudes of 1330-2220 meters in the Shiwalik, Mahabharat and Chulachuli hills of eastern Nepal, Darjeeling district of northern West Bengal and in Bhutan. It inhabits the cloud forests and cool mountain lakes and temporary as well as permanent pools in the eastern Himalayas. However, wherever it is found, it is threatened and its future is uncertain.

The genus *Tylototriton* is presently known by 21 species but the distribution of the Himalayan Salamander is poorly known. Thomas Nelson Annandale (1907-1908), J.C. Daniel (1962) and S.K. Chaudhuri (1966)

were among the first to report the presence of the Himalayan Salamander in the Darjeeling hills of northern West Bengal. P.W. Soman (1966) and Shrestha (1984) noted its distribution in eastern Nepal. Palden (2003) confirmed its presence in Bhutan. The first survey on the species in the Darjeeling hills was conducted by Ritwik Dasgupta during 1983-1988 and he reported the species from 10 sites (Dasgupta, 1990). The second survey was conducted by Daniele Seglie and he reported the species from 16 sites in the Darjeeling hills (Seglie et al. 2003).

Key words:

Amphibia,
bioindicator,
Himalayan Salamander,
sites, *status survey*, *Urodela*.

Tylototriton verrucosus, Manipur.
Photo credit: Shruti Sengupta

Kaushik Deuti

Herpetology Division,
Zoological Survey of India,
27 JL Nehru Road, Kolkata: 700016
Corresponding Author e-mail: kaushikdeuti@gmail.com





Eggs of *Tylototriton himalayanus*.
Photo Credit: M. Firoz Ahmed

Methods

A status survey was conducted by us in the Darjeeling hills of northern West Bengal from June 2005 – July 2007 during the monsoons. The specific small and medium-sized ponds (locally called “pokhries”) which serve as breeding sites for the Himalayan Salamander were visited during six surveys to the area each consisting of 10-15 days duration. The physical parameters of the sites like area, depth, altitude, covering vegetation, surrounding vegetation were noted. The GPS co-ordinates of the sites were also taken. The number of salamanders breeding at each site was also counted.

Results

Our survey revealed the presence of the Himalayan Salamander in 21 wetland sites in the Darjeeling hills of northern West Bengal. All of these sites are located in the Darjeeling and Kurseong sub-divisions of Darjeeling district. Although we visited the Kalimpong sub-division also several times, we did not locate any Salamander populations there. The sites where we located the Himalayan Salamander are described below:

1. **Lake inside Margaret Hope Tea Estate:** It is an oval-shaped mountain lake ($26^{\circ}56'58''\text{N}$ and $88^{\circ}16'58''\text{E}$) with

an area of 3200 square meters at an altitude of 1600 meters. It is situated 2 km down from Gorabari (near Sonada) inside Margaret Hope Tea Estate owned by the Goodricke Tea Company. It has a depth of 2 meters and a few hundred salamanders breed here. The lake is not threatened as it is protected by the Tea Company and the Forest Department.

2. **Reservoir inside Oaks Tea Estate:** It is a rectangular artificial reservoir, earlier constructed for supplying water to the tea estate ($26^{\circ}56'89''\text{N}$ and $88^{\circ}15'19''\text{E}$) with an area of 760 square meters at an altitude of 1560 meters. It is situated 5 km down from Sonada, near Pacheng town, inside Oaks Tea Estate. It has a depth of 1 meter and a few dozen salamanders breed here. However, it is highly threatened by pollution from agricultural pesticides used by the Tea Estate.
3. **Pacheng:** It is a natural marsh but now drying up ($26^{\circ}57'08''\text{N}$ and $88^{\circ}15'87''\text{E}$) with an area of 1540 square meters, at an altitude of 1640 meters. It is situated 4 km down from Sonada beside Pacheng town, the area locally called Pokhrilane. A few scores of salamanders breed in the drains of local houses and maize fields as the

site is threatened by drainage for agriculture and housing.

4. **Reservoir beside Shanti Rani High School (Pacheng):** It is a rectangular artificial reservoir made for storing water (26°59'91"N and 88°15'62"E) with an area of 352 square meters, at an altitude of 1690 meters. It is situated 4 km down from Sonada, beside Shanti Rani High School at Pacheng. A few scores of salamanders breed here but the reservoir is threatened by drainage.
5. **Pool at Maneybhanjang:** This was once a huge marshland which was reclaimed for making the football ground of the High School at Maneybhanjang town (26°59'29"N and 88°07'34"E). The small pool with an area of 10 square meters and the adjoining remnant marshland of 450 square meters is all that is left of the original habitat at an altitude of 1934 meters. Few dozen salamanders breed in this pool which is used for washing utensils and clothes and so gets polluted by detergents. The marshy area is used as a public urinal.
6. **Majhidhura (Near Sukhiapokhri):** It is a natural marshy meadow beside the football ground of the Sukhiapokhri Boys High School (26°52'07"N and 88°15'72"E) with an area of 750 square meters, at an altitude of 2148 meters. It is 25 cm deep and covered with Polygonum herbs. A couple of dozen salamanders breed here but the marshland is threatened by drying (in Gorkha language, Sukhia means drying and pokhri means pond).
7. **Dungdungia (near Sukhiapokhri):** It is a natural marshy place at the top of a hill, 2 km from Sukhiapokhri town near the petrol pump (26°52'12"N and 88°15'73"E) with an area of 600 square meters, at an altitude of 2227 meters. It is 20 cm deep and surrounded by Cryptomeria trees. About a dozen salamanders breed here.
8. **Jorepokhri:** Once two large natural ponds on the top of the Jorepokhri hill (in Gorkha language, Jorepokhri means joined pokhris or ponds) surrounded by Cryptomeria trees (27°02'40"N and



88°15'18"E). The site was declared as India's only Salamander Sanctuary in 1985 by the West Bengal Forest Department. However, the banks of the ponds were cemented and a recreational park build all around which encloses a Tourist Lodge built by the Darjeeling Gorkha Hill Council. Nowadays, salamanders are found in the hill-side forests but cannot breed in Jorepokhri as the breeding habitat has been totally destroyed by artificial construction, release of ornamental fishes and swans in the ponds. One of the ponds is also used for supply of water to the nearby Sukhiapokhri town.

9. **Debrapani:** It is a big natural marshland (26°57'65"N and 88°10'01"E) about 8 km down from Sukhiapokhri towards Pokhriabong. It is almost square-shaped with an area of 2500 square meters, with a depth of 50 cm and covered by grasses and bushes. Situated at an altitude of 1874 meters, a few scores of salamanders breed there. The marsh is not threatened as it is considered as a sacred place by the local people.
10. **Satdobate:** A big natural marshy land (26°57'21"N and 88°10'12"E) and a sacred place for local people on the road from Sukhiapokhri towards Pokhriabong. Area is about 1300 square meters, at an altitude of 1828 meters. A few scores of salamanders were found and they were breeding there too.
11. **Simana:** A small pool (26°55'21"N and 88°08'31"E) beside a permanent stream between Simana and Fatak, surrounded

Tylotriton himalayanus
Photo credit: Shruti Sengupta

by bushes and grasses. This site is just beside the border road with Nepal and less than a dozen salamanders were seen here.

12. **Solimore:** A natural pool (26°55'10"N and 88°08'21"E) between Fatak and Seeyok surrounded by grasses and herbs. Less than 10 salamanders were found to breed in this pool near the Nepal border.
13. **Seeyok:** It is a circular temporary marsh (26°55'59"N and 88°08'48"E) with an area of 112 square meters, at an altitude of 2057 meters. Situated 1 km from Seeyok on the roadside between Sukhiapokhri and Mirik, just opposite to the DGHC view point, near to the border with Nepal. It is 15 cm deep and covered by Polygonum herbs. About half a dozen salamanders breed here but the site is threatened by drying.
14. **Nakhapani Lake (inside Gopal Dhara Tea Estate):** It is a natural lake (26°55'06"N and 88°08'93"E) about 2 km from Seeyok, beside the road between Seeyok and Mirik inside Gopal Dhara Tea Estate. Situated an altitude of 1728 meters, it has an area of 200 square meters, a depth of 60 cm and is

partly covered by *Polygonum* herbs. A few scores of salamanders are found here but the lake is dirty. Local people do not drink the water (in Gorkha language, Na = No, kha = to drink, pani = water).

15. **Ninth Mile – Upper Pond:** It is a circular natural pond at Ninth Mile (26°54'21"N and 88°09'34"E) about 6 km from Mirik, between Seeyok and Mirik. Situated at an altitude of 1614 meters, it has an area of 88 square meters and a depth of 1 meter. About a dozen salamanders breed here but their eggs and larvae are threatened by release of exotic fishes (carps).
16. **Ninth Mile – Lower Ponds:** These are two natural ponds about 1 km from Ninth Mile (26°54'32"N and 88°09'50"E) and about 5 km from Mirik. Situated at an altitude of 1584 meters, they have areas of 78 and 134 square meters and a depth of about 50 cm. About a dozen salamanders breed here but their eggs and larvae are threatened by release of exotic fishes (carps) in these ponds.
17. **Mirik:** The Social Forestry Wing of the West Bengal Forest Department has dug about 20 small artificial pools (each about 2 square meters) at Mirik

A typical Himalayan Salamander habitat in Darjeeling, West Bengal
Photo credit: Abhijit Das



(26°53'14"N and 88°11'23"E) for breeding salamanders at an altitude of 1608 meters. About a hundred salamanders are successfully breeding in them.

18. **Raidhap area above Mirik:** A few dozen salamanders are also found in two small rock pools beside a hill-stream and some rain puddles in the football ground in the Raidhap area above Mirik at an altitude of 1644 meters.
19. **Pokhritar (at Bagora hill):** An oval-shaped natural pond at Pokhritar village (26°58'28"N and 88°07'42"E) with an area of 1750 square meters and a depth of 1.4 meters. Situated 3 km from Bagora at an altitude of 1854 meters, a few scores of salamanders are found here although the lake is threatened by dumping of organic wastes by the villagers.
20. **Chemeli:** A marshy area (26°57'16"N and 88°07'94"E) beside Chemeli village about 7 km from Bagora between Bagora and Dow Hill of Kurseong. It has an area of about 900 square meters at an altitude of 1789 meters. A few dozen salamanders breed at this site.
21. **Namthing Pokhri (near Latpancher):** It is a big oval-shaped natural lake (26°55'81"N and 88°23'98"E) at an altitude of 1437 meters, situated about 5 km from Latpancher. It has an area of 5250 square meters and a depth of 2 meters with a few emergent reeds. A few hundred salamanders breed here as the lake is fenced and hence protected.

Discussion

The Status Survey conducted over three monsoon seasons from 2005-2007 including six visits of 10-15 days duration each, revealed breeding populations of the Himalayan Salamander in a total of 21 breeding sites (small or medium-sized pools, ponds and lakes) in the Darjeeling and Kurseong sub-divisions of Darjeeling district of northern West Bengal State, India. Even small waterbodies with only 15-20 cm of standing water can allow the adults to breed and deposit their eggs preferably on

submerged or emergent vegetation like *Polygonum* spp. Today, due to ever increasing human population, accelerated by the burgeoning tea and tourism industry, all flatlands in the Darjeeling hills are being used for housing and building tourism complexes. The slopes are being converted to huge tea estates. As a result many of the breeding pools have dried up and eggs and tadpoles are stranded during the dry season. It is appalling to see the rapid environmental degradation of hilly areas which were once lush green. Salamanders appear to be sensitive to such human disturbance of their habitat. They do not survive in areas where there has been considerable alteration of habitat due to deforestation and implementation of various developmental projects such as construction of motorable roads. Detergents and agricultural pesticides washed down into the water bodies from the tea gardens by rain causes considerable mortality of developing eggs and larvae. All stages of the life cycle are vulnerable to water pollution because most spend at least part of their lives in water. Many of the smaller water bodies are being used as garbage dumps and public urinals thus totally destroying them as breeding sites for salamanders. Many salamanders also breed in small rock pools that result from spring rains and melting snow. Such pools tend to be especially acidic because humic acid accumulates over winter with each snowfall. A serious threat is the introduction of carps and other exotic freshwater fishes in some ponds which are potential predators of salamander eggs and larvae. Although insignificant when compared to habitat destruction and pollution, exploitation for folk medicine is another threat. The dried and smoked salamander is used as an alleged cure for gastric ailments.

The Himalayan Salamander is rapidly declining in the Darjeeling hills and the future of the species is uncertain as there is no ecological stability. Water bodies which are vital breeding sites for the salamanders should not be drained or filled up. Strong legislation in support of this should be developed. Smaller water bodies should not be used as garbage dumps or public urinals! At certain places, small pools (5 m X 5 m X 1 m) should be dug which can act as

breeding pools for the salamanders. These should have sloping sides, not steep walls. Deforestation of the watershed areas of natural ponds and lakes should be actively prevented. The use of agricultural pesticides in the tea gardens and detergents in households in the hills should be reduced. Carps and other exotic freshwater fishes should not be released into any lakes and ponds between the altitudes of 1300-2200 meters as they destroy salamander eggs and larvae. Developmental projects in the vicinity of such natural wetlands should be stopped immediately. Certain forested areas and wetlands known to support salamander populations need to be specified as sanctuaries. Such wetlands in forested areas and the other important breeding sites mentioned above in this paper, should be fenced and protected as these are the last breeding sites for salamanders in those areas in the Darjeeling Himalaya.

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I am grateful to Dr. Abhijit Das of Wildlife Institute of India for asking me to write this article and to Dr. Kailash Chandra, Director, Zoological Survey of India for permitting me to write it.

Sub-tropical forest beyond
1000m elevation are habitats for
Himalayan Salamander



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Micrixalus saxicola, Brown Dancing Frog, Wayanad, Kerala
Photo Credit : Varun Kher

SECTION

II

BIOLOGY AND BEHAVIOUR

Oviposition sites of some Anurans in East Khasi Hills, Meghalaya, North East India

Abstract

We investigated the oviposition sites and breeding period of 13 species of anurans with special reference to East Khasi Hills of Meghalaya, North Eastern India during the period 2004 - 2015. The 13 species included *Amolops assamensis*, *Euphyctis cyanophlyctis*, *Fejervarya teraiensis*, *Hyla annectans*, *Kaloula pulchra*, *Leptolalax khasiorum*, *Odorrana livida*, *Odorrana mawphlangensis*, *Polypedates himalayensis*, *Polypedates teraiensis*, *Rhacophorus bipunctatus*, *Rhacophorus maximus* and *Xenophrys parva*. Our study therefore identifies the different types of oviposition sites and breeding habitats of the anuran amphibians and helps to understand the importance of such sites. This in turn may help to monitor proper management on preserving such habitats for protecting the amphibian as these animals are facing threats of extinction due to habitat loss and degradation.

Introduction

Oviposition site selection is an important factor that affects the reproductive success of anuran amphibians which breed in a wide variety of aquatic habitats. An appropriate choice of oviposition site is especially critical for oviparous animals that lack parental care (Murphy 2003). For these animals, suitable oviposition sites should protect vulnerable eggs from hydric and thermal stress, predators, and parasites. Several studies indicate that both abiotic and biotic characteristics of the breeding site play an important role in influencing the adult females to choose a potential oviposition site which in turn determines the survival of the offspring (Wells 1977a). Parental care by the adult frogs enhances the survivorship and fecundity of the offspring (McDiarmid 1978). However, in organisms which lack parental care, the survival and growth of the offspring may depend on the quality of the habitat in which their eggs are deposited. Thus, adult frogs are expected to choose habitats that maximize their fitness when potential habitats vary in their suitability for

juveniles. Deposition of eggs by females in unsuitable habitats results in fewer offsprings survival than females that oviposit in suitable habitats; selection of oviposition site by females depends strongly on selective pressure to maximize offspring survival (Crump 1991).

Key words:

Amphibian, breeding habitats, oviposition sites, habitat loss, conservation.

Cherrapunjee plateau of Meghalaya provides ideal breeding habitat for amphibians. Photo Credit: Abhijit Das

Rupa N. K. Hooroo^{1,5}, Ronald K. L. Tron², Duwaki Rangad², Caressa Maebha Thabah¹, Longjam Merinda Devi¹, Shanwell Khongwir³ & Asorphi Janai Iangrai⁴

¹Department of Zoology, North Eastern Hill University, Shillong 793 022, Meghalaya, India

²St. Edmund's College, Shillong 793003, Meghalaya, India

³Shillong College, Shillong 793003, Meghalaya, India

⁴Synod College, Shillong 793002, Meghalaya, India

⁵Corresponding author e-mail: rnkhnehu@hotmail.com





Meghalaya state in North East India is a part of the Global biodiversity hotspot. Amphibian research from this region is largely included survey and taxonomy (Roonwal and Kripalani 1961; Yazdani and Chanda 1971; Pillai and Yazdani 1973; Pillai and Chanda 1977, 1979; Sahu and Khare 1983; Hooroo et al. 2002; Sen 2004; Rangad et al. 2007; Das et al. 2009 and 2010) and few on breeding biology and development (Roy 1979; Khongwir 2004, 2016; Iangrai 2007; Rangad 2014 and Tron 2014). The present study emphasizes the importance of the breeding habitat and oviposition site of these anurans in Meghalaya. Obtaining accurate information on amphibian breeding habitats and oviposition sites can be challenging as many species have different breeding biology requiring different habitat for breeding and oviposition. Presently, amphibian species all over the world are experiencing significant threats extinction due to habitat loss and degradation. In East Khasi Hills of Meghalaya the major forms of habitat destruction are in the form of mining (sand, limestone and coal), quarrying, constructions and forest fires. Identifying their breeding habitats and oviposition sites has now become necessary to understand how these changes affect habitat suitability for different anuran species. Proper management targeted at enhancing amphibian breeding areas and oviposition sites can help in protection and

conservation of the amphibian species.

Materials and Methods

Survey was conducted in different areas of East Khasi Hills, Meghalaya, North Eastern India from the year 2004 – 2015. The location (latitude/longitude) and elevation of the surveyed areas were determined with the help of Garmin (etrex) Global Positioning System (GPS). Survey was conducted in the afternoon from 2:00 PM and continued till evening depending on their breeding season. After identification of the sampling site, survey period at each selected site was covered at different times of the day in order to record the breeding period of the selected species. All the species have been identified by comparing their measurements and morphological characters with the description of their respective holotype and available published literatures (Chanda 1994; Sengupta et al. 2008; Das et al. 2010). Audio Encounter Surveys (AES) and Visual Encounter Surveys (VES) were used to identify exact locations where adult frogs are attempting to breed. Egg mass and larval surveys provide evidence about the oviposition site and the breeding period of the species. Torch light, headlamp and bamboo torch were used to locate and count the number of males and females of the frogs at their breeding sites during night surveys. Field sampling was carried out daily during the

Hyla annectans.
Photo Credit: Abhijit Das

breeding season and at weekly intervals during the non- breeding period with each sampling session spanning over two to three continuous days. All photographic documentation was made using digital SLR (Nikon D 3200).

Results

Oviposition sites of the 13 anuran species of East Khasi Hills (*Amolops assamensis*, *Euphlyctis cyanophlyctis*, *Fejervarya teraiensis*, *Hyla annectans*, *Kaloula pulchra*, *Leptolalax khasiorum*, *Odorrana livida*, *Odorrana mawphlangensis*, *Polypedates himalayensis*, *Polypedates teraiensis*, *Rhacophorus bipunctatus*, *Rhacophorus maximus* and *Xenophrys parva*) were observed (Table 1) during the study from different types of breeding habitats and a description of the oviposition site of each anuran species is described below:

Oviposition site of *Amolops assamensis*

The oviposition site of *Amolops assamensis* (Figure 1a) was observed in a fresh water stream in Nongspung village (25°27' N; 91°36' E; 1644m.asl) located about 50 km. away from Shillong. *Amolops assamensis* a large frog where snout vent length ranges from 53-90 mm (Sengupta et al. 2008) breeds during the months of February to March in a flowing stream a short distance away from Nongspung village. This species was first described by Sengupta et al. (2008) from the splash zones of moist dark crevices in a fast flowing stream in Assam. It was observed that the adults of *Amolops assamensis* emerge as early as in the month of January and they come to breed in the same spot of the stream every year during the study period (2009-2012). The oviposition site was observed to be on the



Figure 1a : A fresh water stream in Nongspung village. Meghalaya

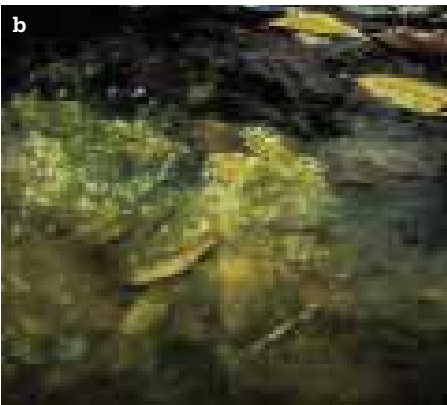


Figure 1b : The oviposition site of *Amolops assamensis*.



Figure 1c : The female *Amolops assamensis* lay egg masses that are attached to the surface of the rock walls in the breeding habitat

side of a flowing stream that cascades into a deep pool (Figure 1b). This stream was lined with rocks and pebbles, mostly covered by green moss where the frogs come out to breed. Adults have been observed to show aggregation behaviour during the breeding period. The females lay egg masses that are attached to the surface of the rock walls in the breeding habitat about 2 to 3 feet below the water (Figure 1c).

Oviposition site of *Euphlyctis cyanophlyctis*

Oviposition sites of Indian skipper frog, *Euphlyctis cyanophlyctis* (SVL 31-61 mm) (Figure 2a) found to be temporary and permanent water bodies such as, ponds,

muddy pools and marshes of Cherrapunjee (25°18'N; 91°42'E; 1484m.asl), Myliem (25°30'N; 91°49'E; 1625m.asl) and NEHU campus, Shillong (25°36'N; 91°53'E; 1418m.asl) (Figure 2b). It is a seasonal breeder and its breeding activity coincides with the monsoon season i.e. March up to August. In addition, it was also observed that the frog would deposit its eggs on floating algae and among submerged vegetation on the edges of standing water bodies (Figure 2c). The eggs deposited were scattered and spread over the water surface as depicted. *Euphlyctis cyanophlyctis* exhibits aquatic oviposition as the eggs are laid in the water body.



Figure 2a : Indian skipper frog, *Euphlyctis cyanophlyctis* adult male.

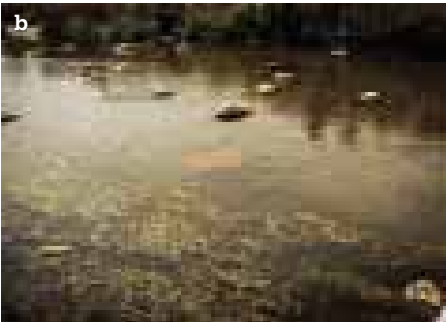


Figure 2b : Habitat of Indian skipper frog, *Euphlyctis cyanophlyctis*.



Figure 2 c : Eggs deposited by Indian skipper frog, *Euphlyctis cyanophlyctis*.

Oviposition site of *Fejervarya teraiensis*

The oviposition of *Fejervarya teraiensis* (SVL 35.7-52.3 mm) (Figure 3a) was documented at Malki forest, Shillong (25°35'N; 91°55'E; 1500-1800m.asl) and Laitkroh community forest (25°26'N; 91°48'E; 1610m.asl). *Fejervarya teraiensis* found to select rain fed pools that are permanent or semi permanent, rain water puddles, marshes and ditches (Figure 3b). *Fejervarya teraiensis* breeds throughout the months of

April to July. The oviposition sites are surrounded by vegetation which provides ideal hiding place for the adult frogs. Breeding activity starts once the pools and pond gets filled with rain water. The females deposit their eggs in these aforementioned sites and the eggs can be found floating on the surface of the water (Figure 3c). The eggs are pigmented and have a thin layer of jelly cover. The tadpoles were observed to swim freely in the water column or were swimming close to margin of the ponds (Figure 3d).

Figure 3 a : Amplexus in *Fejervarya teraiensis*



Figure 3 b : Habitat of *Fejervarya teraiensis*



Figure 3c : Eggs deposited by female of *Fejervarya teraiensis*.



Figure 3d : Tadpoles of *Fejervarya teraiensis*.

Oviposition site of *Hyla annectans*

Hyla annectans (SVL 23-48 mm) (Figure 4a), the Indian hyloid frog was collected from Myllem (25°30'N; 91°49'E; 1625m.asl) as well as the grassland areas of Cherrapunjee (25°18'N; 91°42'E; 1484m.asl). In Meghalaya, *Hyla annectans* found to breed during the month of March till June during the early part of the monsoon season. However, Ao and Bordoloi (2000) reported that this species breeds during the month of May to July in Nagaland. In the present study it was observed that this species breeds in temporary ponds, rainfed pools, puddles and waterlogged terraced paddy fields at forest edges (Figure 4b). A unique feature about this frog is that it selects its oviposition site only on pristine and clean water bodies as was observed in the instant case in the forest edges which are free from any anthropogenic disturbances. Adult female of

Hyla annectans chooses to oviposit its eggs on submerged vegetation (Figure 4c). The eggs which are covered with thick jelly stick to one another and float as a mass on the water. *Hyla annectans* thus shows aquatic oviposition which is a feature of most anuran amphibians.



Figure 4a : Amplexus in *Hyla annectans*.



Figure 4b : Habitat of *Hyla annectans*.

Figure 4c : Eggs deposited by female *Hyla annectans*

Oviposition site of *Kaloula pulchra*

During the present survey, the oviposition site of *Kaloula pulchra* (SVL 60.5-64.21 mm) (Figure 5a) was studied at Laitkynsew village (25°13'N; 91°39'E; 915m.asl) and Cherrapunjee (25°18'N; 91°42'E; 1484m.asl) East Khasi Hills, Meghalaya. *Kaloula pulchra* select standing water bodies like cemented tanks, rock pools, rain fed pools as their breeding habitats (Figure 5b) during the months of April to July. *Kaloula pulchra* was commonly found in artificial tanks or wells which are used by the villagers for either storing water or most usually for fermenting “kwai” or the betel nut (*Areca catechu*) (Figure 5c). During the monsoon period these tanks or wells get filled with

water. The villagers dip sacks of *Areca catechu* in these wells for months and leave them there undisturbed. In such water tanks, the adult frogs of *Kaloula pulchra* were found to deposit their eggs on the surface of the water. These pigmented eggs which are covered with thin jelly covering float on the water surface.



Figure 5a: Oviposition site of *Kaloula pulchra*.



Figure 5b: Breeding habitat of *Kaloula pulchra*.

Figure 5c: Eggs in the habitat of *Leptolalax khasiorum*

Oviposition site of *Leptolalax khasiorum*

Das et al. (2010) described *Leptolalax khasiorum* (SVL 25.36-31.89 mm) from a forest stream in Mawphlang (25°26'N; 91°44'E; 1813m.asl). Tron et al. (2015) observed that the breeding period of this frog lasts for a very short time i.e. from the month of February to March (Figure 6a) and adults can be found till the month of April. It was observed and reported that the adult

frogs prefer to remain hiding under rocks and are found after lifting rocks along the stream bed during daytime. Since these frogs emerge in early spring, sometimes even before the rain sets in, the only choice as breeding areas are mostly confined to small pockets of water or puddles in the rocky stream bed (Figure 6b). These water pools are rich in organic debris derived from leaf litter along with mud and sand. Females lay egg masses that are usually attached to rocks, leaves sometimes even

on the underside of rocks in the stream bed and on the edges of the stream (Tron et al. 2015) (Figure 6c). Eggs are small and white and covered with a thin transparent jelly (Figure 6d).



Figure 6a: *Leptolalax khasiorum* in its habitat.



Figure 6b: Habitat of *Leptolalax khasiorum*.

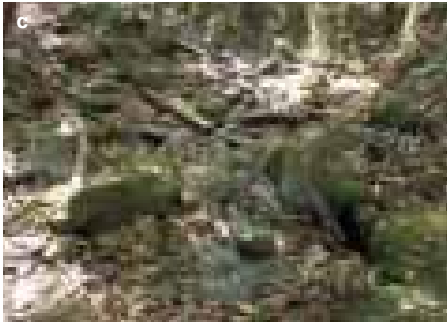


Figure 6c, 6d: Habitat of *Leptolalax khasiorum*.

Oviposition site of *Odorrana livida*

Observation on oviposition of *Odorrana livida* (SVL 68.63- 97.44 mm) was made from forest streams at Riat Laban (25°36'N; 91°53'E; 1500-1800 m.asl) and Malki forest (25°35'N; 91°55'E; 1500-1800m.asl). Adults of *Odorrana livida* (Figure 7a) have been seen to emerge in the breeding habitat during the months of May to July. The adults have been observed to breed in stream section having small collection of stagnant water pool surrounded by riparian vegetation. The stream bank was observed to have earth or

rock walls with small crevices in them, from where the adult frogs have frequently been seen to emerge (Figure 7b). These frogs do not breed randomly throughout the stream. Females lay egg masses that are submerged in water along the side of the stream and in some cases attached to wet rocks and pebbles along the forest stream (Figure 7c). The eggs are individually covered with jelly layers and are attached to each other. The eggs form a multitier layer to form a clump.



Figure 7a: *Odorrana livida* in its Habitat



Figure 7b: Habitat of *Odorrana livida*.

Figure 7c: Egg deposition site of *Odorrana livida*.

Oviposition site of *Odorrana mawphlangensis*

The oviposition site of *Odorrana mawphlangensis* (SVL 61.93-94.97 mm) was studied in a forest stream at Mawphlang (25°26'N; 91°44' E; 1830m.asl). *Odorrana mawphlangensis* (Figure 8a) was observed to start emerging in the habitat from late April onwards. Their breeding activity starts from May and last till July or early August. It was generally observed that the calling males of this species start emerging first. *Odorrana mawphlangensis* used specific oviposition sites in the stream during the period of investigation (Figure 8b). The oviposition site is a pool of water with a soft substratum of mud and organic debris. The selected stream had half submerged boulders that formed cave-like spaces which were used as oviposition sites (Figure 8c). These specific sites are seen at dark and damp places with a canopy cover. Adults have been observed to emerge from the holes on the sides of streams about 1.5 to 3 metres above the stream near the oviposition site. The female usually lays egg masses directly on the substratum of the

stream or attached to rock walls on the side of the stream and the egg mass is always submerged inside the water (Figure 8c). It was observed that each of the individual egg is wrapped in a semi-transparent jelly in the egg masses.

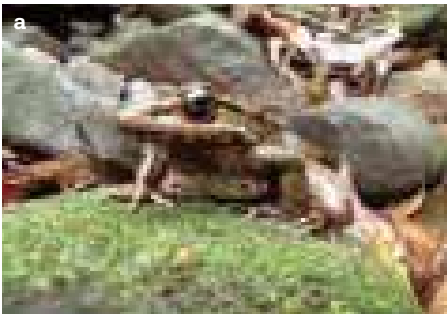


Figure 8a: *Odorrana mawphlangensis* in its Habitat.

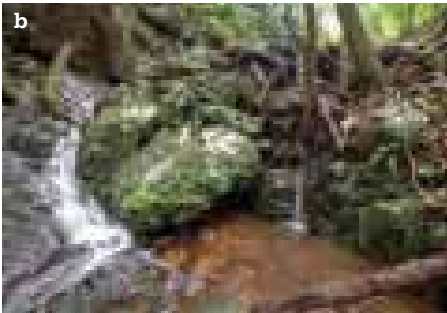


Figure 8b: habitat of *Odorrana mawphlangensis*.



Figure 8c : Egg deposition site of *Odorrana mawphlangensis*.

Oviposition site of *Polypedates himalayensis*

During the present investigation, it was observed that *Polypedates himalayensis* (SVL 41.0- 62.0 mm) (Figure 9a) is a seasonal breeder and its breeding activity coincides with the onset of few showers of rainfall i.e. from March to July. The study was conducted at an agricultural field (Figure 9b) which is adjacent to a community forest located at a village named Myllem (25°30'N; 91°49'E; 1625m.asl) about 18 km. from Shillong, East Khasi Hills District. During the study, it was found that *Polypedates himalayensis* selects its oviposition site near standing water bodies. Females of *Polypedates himalayensis* lay its eggs by constructing foam nests half a meter or in some cases even one meter away from waterbody (Figure 9c). The construction of foam nest was found to occur inside earthen holes which are damp, moist and also under thick vegetation cover.

Foam nests of *Polypedates himalayensis* were also collected from a pond located at North Eastern Hill University (NEHU). In such built up habitats *Polypedates himalayensis* constructs foam nests under moist or wet planks, logs of wood and also under vegetation (pine leaves of *Pinus khasiana*) (Figure 9d). Spawning and foam nest construction started from the month of April and was seen to last till the month of July.



Figure 9a : Amplexus in *Polypedates himalayensis*.



Figure 9b : Habitat of *Polypedates himalayensis*.

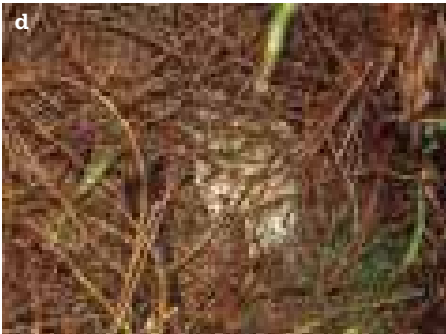
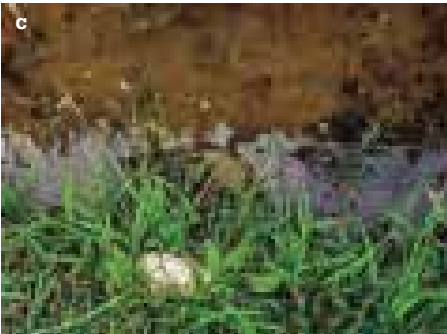


Figure 9c : Females of *Polypedates himalayensis* lay its eggs by constructing foam nests nearby waterbody.

Figure 9d : *Polypedates himalayensis* constructs foam nests under moist or wet planks, logs of wood and also under vegetation.

Oviposition site of *Polypedates teraiensis*

Polypedates teraiensis (SVL 42.0-78.0mm) (Figure 10a) or the common tree frog is a seasonal breeder and its breeding activity coincides with the onset of monsoon. The breeding period of this species was found to last from March to early September. In the present study, it was seen that *Polypedates teraiensis* is commonly found in many grassland pools located at Cherrapunjee (25°18'N; 91°42'E; 1484m.asl) and many foam nests were observed in these grassland pools during the breeding season (Figure 10b). Further it was observed that *P*

teraiensis is syntopic with *P. himalayensis*. This tree frog selects its oviposition sites close to water bodies and sometimes lays its eggs in vegetation cover above water. In this case the oviposition is aquatic and it occurs in standing water of the grassland pools. Apart from construction of foam nests in grassland vegetation, the study also revealed that *Polypedates teraiensis* sometimes chooses to construct its foam nest in cemented tanks about 15-30 cm above water in Cherrapunjee (Figure 10c) and also on small rocks and stones on or near temporary pools (Figure 10d).

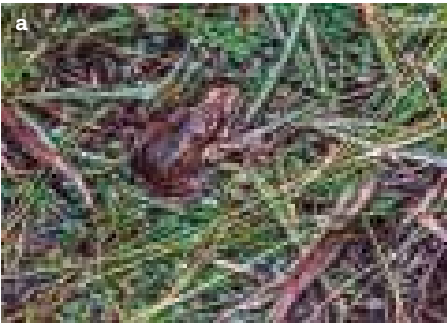


Figure 10a : Amplexus observed in *Polypedates teraiensis*.

Figure 10b : Foam of *Polypedates teraiensis* nests were observed in these grassland pool.

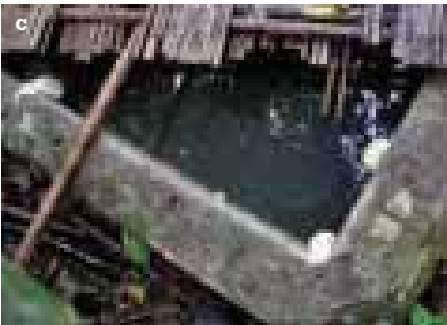


Figure 10c : *Polypedates teraiensis* sometimes chooses to construct its foam nest in cemented tank.

Figure 10d : Eggs deposited by *Polypedates teraiensis* stones on or near temporary pools

Oviposition site of *Rhacophorus bipunctatus*

Rhacophorus bipunctatus (SVL 39.0-65.0 mm) (Figure 11a) or the twin spotted tree frog is also a seasonal breeder with its breeding period coinciding with the onset of monsoon i.e. from the month of March to August. The present study recorded the occurrence of this species from Cherrapunjee, Laitkynsew Village (25°13' N; 91°39' E; 915m.asl) and NEHU Campus Shillong. During the present investigation, it was found that *Rhacophorus bipunctatus* chooses its

oviposition sites on overhanging vegetation above water bodies (Figure 11b). It was observed that this frog constructs small foam nests (6-8 cm in diameter) on leaves of trees, bushes and grasses that are close to about 1 m approximately above water bodies (Figure 11 c). Oviposition sites were also documented from tree holes and walls of cemented water tanks at Laitkynsew village (Figure 11 d). Apart from selecting overhanging vegetation to deposit its eggs, *Rhacophorus bipunctatus* also construct its foam nests on the ground surface covered by grasses or leafy vegetation either close to a water body or away from it.

Figure 11a: Amplexus observed in *Rhacophorus bipunctatus*.



Figure 11b: Egg deposition site of *Rhacophorus bipunctatus*.

Figure 11c: Egg deposition site of *Rhacophorus bipunctatus*.



Figure 11d: : Egg deposition site of *Rhacophorus bipunctatus*.

Oviposition site of *Rhacophorus maximus*

Rhacophorus maximus (SVL 35.0- 68.0 mm) (Figure 12a), the large tree frog was found in many grasslands and forest covers of Cherrapunjee (25°16 N; 91°44 E; 1484m.asl) as well as Mawsynram village (25° 18' N; 91° 35' E; 1400m.asl). Khongwir et al. (2016) reported that the breeding period begins with the onset of first shower of rainfall during the month of March and lasts till early May. This tree frog selects it oviposition site on grassy vegetation near shallow temporary rainfed pools and the female constructs large foam nests on such

vegetation which then appears to be floating on the water surface (Figure 12b and 12c). In addition *Rhacophorus maximus* also chooses to oviposit its eggs on vegetation above water surface, on earthen banks attached to grasses and stones on the edges of the pond (Figure 12d). Such temporary rainfed standing water serves as a potential breeding site for this frog and since these temporary pools dry up fast so also the breeding period of this frog is also very short. Therefore, from the study it is clear that *Rhacophorus maximus* exhibits aquatic oviposition and constructs foam nests on any substrata where water is available.

Figure 12a: An adult individual of *Rhacophorus maximus* documented from cherapunjee, Mwghalaya.

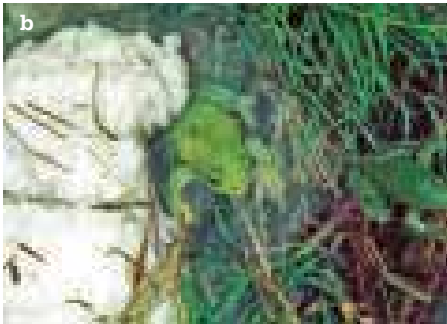


Figure 12b: Female *Rhacophorus maximus* is laying eggs in the edges of the temporary pools.

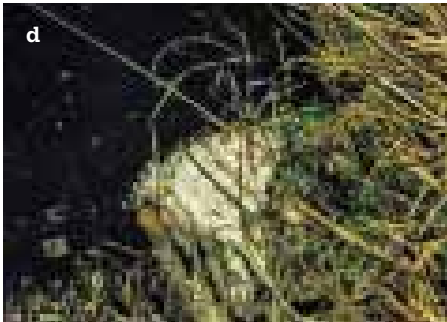


Figure 12c: Foam nest of *Rhacophorus maximus* near pool edge.

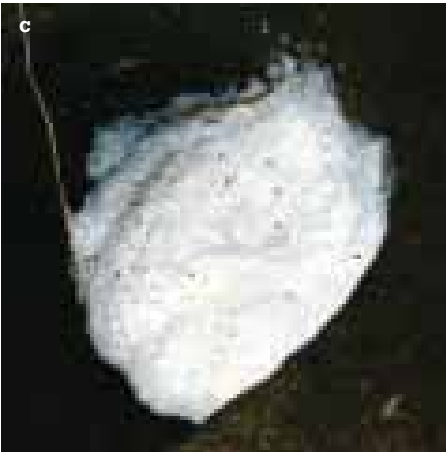


Figure 12d: *Rhacophorus maximus* also chooses to oviposit its eggs on vegetation above water surface, on earthen banks attached to grasses and stones on the edges of the pond.

Oviposition site of *Xenophrys parva*

From this study it was observed that *Xenophrys parva* (SVL 31.62-39.27 mm) (Figure 13a) is a monsoon breeder that comes out to breed during the months of April to July. The breeding habitat of *Xenophrys parva* at Mawphlang (25°26'N; 91°44'E; 1814m.asl) is a forest stream (Figure 13b). Since this species emerges during the monsoon, there is abundant water in the stream around the time they emerge. Shallow pools are formed along the side of the stream with sand, stone and organic debris on the stream bed. The stream has

sloping earth walls on both sides of the banks with rocks and boulders present along the stream. The vegetation growing along riparian area creates shaded pool with floating and submerged organic debris such as leaves, pollen, flowers, etc. *Xenophrys parva* has been observed to be found in the portions of the stream that is associated to vegetation growing close to the stream and sometimes overhanging it. The females of *Xenophrys parva* chooses to oviposit its eggs on the moist decaying leaf litter and organic debris present along the edges of the stream as well as on small stones and pebbles adjacent to the stream (Figure 13c and 13d).

Figure 13a: An adult individual of *Xenophrys parva* documented from Mawphlang, Meghalaya.

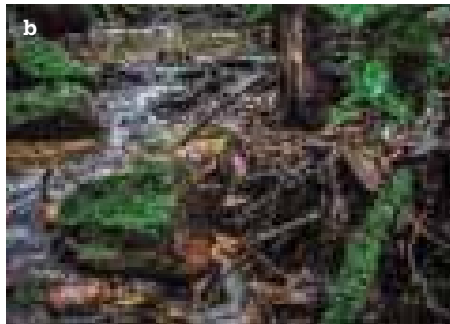


Figure 13b: Habitat of *Xenophrys parva*.

Figure 13c: The females of *Xenophrys parva* chooses to oviposit its eggs on the moist decaying leaf litter and organic debris present along the edges of the stream as well as on small stones and pebbles adjacent to the stream.



Figure 13d: Egg laying site of *Xenophrys parva*.

Anuran Species	Area	GPS location	Altitude (m.asl)	Breeding Habitats	Oviposition Sites
<i>Amolops assamensis</i> (Family- Ranidae)	Nongspung	25°27'N; 91°36'E	1644	Flowing stream	Lays eggs inside the water body.
<i>Euphlyctis cyanophlyctis</i> (Family-Dicroglossidae)	Cherrapunjee	25°18'N; 91°42'E	1484	Temporary as well as permanent standing water bodies, marshes, ponds and puddles.	Deposits eggs amidst aquatic plants on the muddy pools and marshes also on floating algae present on the edges of standing water bodies.
	Myllem	25°30'N; 91°49'E	1625		
	NEHU Campus Shillong	25°36'N; 91°53'E	1418		
<i>Fejervarya teraiensis</i> (Family- Dicroglossidae)	Malki Forest; Laitkroh community forest	25°35'N; 91°55'E 25°26'N; 91°48'E	1500-1800 1610	Permanent or semi permanent habitat with a lentic ecosystem like a pond, rain water puddles.	Lays eggs on water surface.
<i>Hyla annectans</i> (Family-Hylidae)	Myllem	25°30'N; 91°49'E	1625	Temporary ponds, rainfed pools, puddles and terraced paddy fields located at the edge of forests where water logging is observed.	Oviposit eggs on vegetation that is submerged in water
	Cherrapunjee	25°18'N; 91°42'E	1484		
<i>Kaloula pulchra</i> (Family- Microhylidae)	Laitkynsew village Cherrapunjee	25°13'N; 91°39'E 25°18'N; 91°42'E	915 1484	Non-permanent and standing water bodies like cemented tanks, rock pools and rain fed pools.	Eggs deposited on the water surface.
<i>Leptotalax khasiorum</i> (Family- Megophryidae)	Mawphlang	25°26'N; 91°44'E	1813	Forest stream, breeding areas mostly confined to small pockets	Females lay egg masses that are usually attached to rocks or leaves, sometimes even

Anuran Species	Area	GPS location	Altitude (m.asl)	Breeding Habitats	Oviposition Sites
				of water or puddles that remains in rocky stream bed.	on the underside of rocks.
<i>Odorrana livida</i> (Family- Ranidae)	Riat Laban Reserve Forest	25°36'N; 91°53'E	1500-1800	Fast flowing forest stream, cascades.	Eggs laid are submerged in water and in some cases attached to wet rocks and pebbles along the forest stream.
	Malki Forest	25°35'N; 91°55'E			
<i>Odorrana mawphlangensis</i> (Family- Ranidae)	Mawphlang Sacred Grove	25°26'N; 91°44' E	1830	Forest stream.	Lay egg masses directly on the substratum or attached to rock walls and the egg masses are always submerged inside the water.
<i>Polypedates himalayensis</i> (Family- Rhacophoridae)	Mylliem	25°30'N; 91°49'E	1625	Agricultural field, perennial pond.	Lays eggs by constructing foam nests away from the water body, in earthen holes, under wet planks and covered vegetation.
	NEHU Campus Shillong	25°36'N; 91°53'E	1418		
<i>Polypedates teraiensis</i> (Family- Rhacophoridae)	Cherrapunjee	25°18'N;91°42'E	1484	Grassland pools, rain fed pools and cemented tanks.	Foam nest construction on vegetation above water surface, on surfaces of rocks close to water body and walls of water tanks.
<i>Rhacophorus bipunctatus</i> (Family- Rhacophoridae)	Cherrapunjee	25°18'N;91°42'E	1484	Forest, bushes, tree holes, water tanks, rainfed pools.	Constructs small foam nests on leaves of trees, bushes, grasses and tree holes that are close to or few meters above water bodies and also in walls of cemented water tanks.
	Laitkynsew village	25°13'N; 91°39'E	915		
<i>Rhacophorus maximus</i> (Family- Rhacophoridae)	Cherrapunjee	25°16'N;91°44'E	1484	Temporary shallow rainfed pools	Constructs foam nest on grassy vegetation that has been covered with water, on earthen banks attached to grasses and stones and any substrata where water is available.
	Mawsynram	25° 18' N;91° 35' E	1400	Temporary rainfed pond	
<i>Xenophrys parva</i> (Family- Megophryidae)	Mawphlang	25°26'N; 91°44'E	1814	Edges of forest stream accumulated with moist leave litter, organic debris, stones and pebbles.	Oviposition takes place on moist decaying leave litter and organic debris present along the edges of forest stream as well as on small stones and pebbles adjacent to the stream.

Discussion

Anuran amphibians occupy and deposit their eggs in diverse habitats (Resetarits Jr. 1996). Many anuran species require water for successful oviposition. The diversity of aquatic habitats in which anurans can choose, range from shallow pools, rainfed puddles, cemented tanks, small temporary ponds to large permanent ponds, lakes, mountain streams, and rivers. Anurans clearly show selective preference for different habitats for oviposition. Anuran species that typically breeds in standing water is unlikely to oviposit its eggs in fast flowing stream. Different anuran species are expected to exhibit active selection of oviposition site rather than deposit their eggs in the environment at random, because offspring survival is strongly dependent on characteristics of oviposition site. These characteristics include physical parameters such as water depth (Crump 1991), size of the site, water temperature (Herreid and Kinney 1967; Howard 1978, 1980; Seale 1982; Waldman 1982; Caldwell 1986), and vegetation structure (Wells 1977b and Howard 1978), as well as biotic features such as potential predators and competitors (Resetarits and Wilbur 1989 and Laurila and Aho 1997). The oviposition site that the adult anuran selects considerably influences hatching success, larval performance, and in turn affects the parental fitness. Therefore, the ability of the adult anurans to select suitable oviposition sites on the basis of expected larval performance should be strong. This is particularly true when the respective larval stages cannot migrate to better habitat patches without involving high expenditure of energy which in turn increases rate of larval mortality.

In our investigation of oviposition site selection by anurans in East Khasi Hills, each species of anuran was found to select a unique site for oviposition. All the thirteen (13) species observed during the present survey including *Amolops assamensis*, *Euphyctis cyanophlyctis*, *Fejervarya teraiensis*, *Hyla annectans*, *Kaloula pulchra*, *Leptolalax khasiorum*, *Odorrana livida*, *Odorrana mawphlangensis*, *Polypedates himalayensis*, *Polypedates teraiensis*, *Rhacophorus bipunctatus*, *Rhacophorus*

maximus and *Xenophrys parva* selected aquatic habitats or a site close to a water body for deposition of the eggs. Some species like *Amolops assamensis*, *Odorrana livida*, *Odorrana mawphlangensis*, *Leptolalax khasiorum* and *Xenophrys parva* selected forest stream for oviposition. While species like *Euphyctis cyanophlyctis*, *Fejervarya teraiensis*, *Hyla annectans*, *Kaloula pulchra*, *Polypedates himalayensis*, *Polypedates teraiensis*, *Rhacophorus bipunctatus* and *Rhacophorus maximus* selected standing water bodies that are permanent or non permanent. The most generalized mode of reproduction which is commonly found in about 80% of the anuran families is the oviposition in standing water (Duellman and Trueb 1986 and Wells 2007).

Polypedates himalayensis, *Polypedates teraiensis*, *Rhacophorus bipunctatus* and *Rhacophorus maximus* of the family Rhacophoridae (tree frogs) deposited their eggs in a foam nest on vegetation close to water bodies. *Xenophrys parva* of the family Megophryidae also deposited egg mass attached to rocks or vegetation close to a water body. The deposition of eggs in a foam nest away from the water body may provide protection to the embryos at the early stages from predation (Mohanty-Hejmadi and Dutta 1988). Similarly, Duellman and Trueb (1986) suggested that when eggs are laid away from water in a foamy mass, the tadpoles develop to a pre-metamorphic stage before falling into water which may be an alternative life history strategy of anurans. Hodl (1992), Magnusson and Hero (1991), considered this strategy to facilitate predator avoidance of eggs and early-stage tadpoles, and to reduce the duration of the larval stage by rapid development during the out-of-water phase. A common suggestion is that foam nests protect the eggs from dessication (Salthe and Mecham 1974; Duellman and Trueb 1986; Hodl 1986).

In the present investigation it was observed that egg masses of *Odorrana mawphlangensis* and *Odorrana livida* were similar as in both cases the eggs are non pigmented and are deposited as a clump in water. Aquatic and terrestrial clumps generally forms a multi-tiered stack that lack a common, surrounding surface or

matrix with interstices among eggs and the adjacent jellies remain distinct even if melded (Altig and McDiarmid 2007), as can be seen in the clumps of *Odorrana mawphlangensis* and *Odorrana livida*. Egg masses in *Leptolalax khasiorum*, *Xenophrys parva* and *Amolops assamensis* also deposited non-pigmented eggs and covered by a transparent jelly. Eggs deposited in hidden locations often are unpigmented as oviposition sites protect the eggs and minimize ultraviolet radiations (Salthe and Mecham 1974). According to various workers, some of the functions of egg jellies include mechanical support for the ovum, attachment of eggs to each other or a structure in the environment (Greven 2002, 2003); enhancement or prevention of entry by conspecific and heterospecific sperm respectively (Barbieri and Del Pino 1970); prevention of polyspermy, sperm capacitation, differential protection from water molds like *Saprolegnia* and *Achlys* (Gomez-Mestre et al. 2006); protection from contaminants (Marquis et al. 2006); and protection from predators, pathogens, and environmental stressors such as temperature and UV light (Hunter and Vogel 1986; Itoh et al. 2002; McLaughlin and Humphries 1978; Ward and Sexton 1981). It was observed that *Fejervarya teraiensis*, *Euphlyctis cyanophlyctis*, *Kaloula pulchra* deposited pigmented eggs that float on the surface of the water. Eggs that are laid in exposed areas usually have melanic pigment at the animal pole (e.g., *Ambystoma*, *Bufo*, *Hyla*, and *Rana* of North America) regardless of the specific site, taxon, or ovipositional mode (Altig and McDiarmid 2007). The eggs of *Hyla annectans* were also slightly pigmented and found to be floating on the surface of the water. However, *Hyla annectans* was observed to display a unique behavior in selecting only clean and pristine undisturbed sites for oviposition of the eggs.

In addition to this, it was observed that *Odorrana mawphlangensis*, *Odorrana livida* and *Amolops assamensis* returned to the same breeding site for oviposition each year. This behaviour is termed as site fidelity, whereby individuals tend to return to a previously occupied site (Bucciarrelli 2016). It is presumed that individuals of these species may have site fidelity but mark

recapture study was not done in the present investigation. Hence, further studies may be carried out by marking the individuals during the breeding season to prove if they returned back to the same spot for breeding in these specific microhabitats. Thus, implementation of conservation measures of these oviposition sites of amphibians may be considered to be significant. Some amphibians have been reported to display site fidelity to the breeding site, where they often remain in that microhabitat during the entire breeding period (Packer 1963; Crump 1986). Liao (2011) also documented the site fidelity of *Amolops mantzorum* in a montane region in China. Site fidelity may be related to territoriality (Crump 1986) and can be displayed by male frogs that reside for several nights or weeks at the oviposition site to defend against conspecific intruders, which may be a pattern that increases the territorial male's success in attracting females who lay eggs in his territory (Wells 1978); or territoriality displayed by female frogs for sites that can provide high-quality oviposition environment and abundant food resources (Wells 1977b). According to Crump (1986), anurans can also return to the same site due to ideal microhabitat conditions in terms of moisture, abundance of crevices in which to hide and sufficient overhanging vegetation to provide protection from the sun, other than food.

It may be mentioned that parental care was observed, in the form of male remaining at the oviposition site for a few days after oviposition, in some of the anuran species like *Amolops assamensis*, *Leptolalax khasiorum*, *Polypedates himalayensis* and *Rhacophorus maximus*. Similar observations in males of *Colostethus subpunctatus* has also been reported to remain with the egg masses until they hatch (Stebbins & Hendrickson 1959). According to Wells (1977b), male parental care in many anurans is probably an outgrowth of territorial defence of oviposition sites by males which in turn is related to external fertilization of eggs. Hence a male defending a suitable oviposition site might care for eggs already laid while continuing to attract additional females into his territory (Trivers 1972).

During the period of our investigation (2004 to 2015) in East Khasi Hills of Meghalaya, a general trend of degradation of habitats was observed throughout the different surveyed areas. In due course of time, suitable breeding sites were transformed into non suitable breeding or oviposition sites for anurans. This can be attributed to the large number of disturbances in the areas in the form of clearing of forests for constructions of roads, buildings and for agricultural purposes. This resulted in loss of many breeding habitats that affected the oviposition site selection of many anurans, especially in species that have a strong preference for a site like *Hyla annectans* (that required undisturbed areas). Species that are more adaptive like *Rhacophorus bipunctatus* and *Polypedates himalayensis* were observed to move to other similar suitable sites nearby. Therefore, conservation of breeding habitats becomes

very important for anuran species that show a strong preference for the specific oviposition sites. It may be mentioned that the role of the community has been seen to be very important in the conservation process. Anuran species that selected undisturbed areas for oviposition like *Amolops assamensis*, *Odorrana livida*, *Odorrana mawphlangensis*, *Leptolalax khasiorum* and *Xenophrys parva* were all found in community reserved forests. Community forests are protected through prohibition of human activities due to some cultural or religious beliefs, and therefore these forests remain undisturbed. Coincidentally, these forests serve as good habitats for these anuran species and they remain protected. Hence these cultural practices play a major role in the conservation of the amphibian habitats.

Amphibian breeding habitats are often polluted by improper garbage disposal.



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Retinoids and Tail regeneration in frog tadpoles

Abstract

Regeneration is the process of restoration of lost parts of an organism and is wide spread in animal kingdom. Amongst vertebrates, the tadpoles of anuran amphibians regenerate their body parts like limbs and tails. Tadpole is not only an ideal model to study regeneration of organs but also ideal for understanding loss of regenerating capacity as it stops regeneration of organs soon after onset of metamorphosis. Tail amputated tadpoles treated with vitamin A / retinoic acid show an interesting phenomenon of homeotic transformation where tail cells are transdifferentiated into cells of ectopic pelvic girdle and hind limbs. In this review, effects of vitamin A /retinoic acid on tail amputated tadpoles of different species of Indian anurans has been described. Vitamin A treatment leads to regeneration of an abnormal tail in the tail amputated tadpoles. The ectopic hind limbs developed at the cut end of tail follow the path of normal hind limb development. Vitamin A treatment induces oxidative stress in the tail regenerates. Specific activities of two phosphatases, namely acid and alkaline phosphatase are elevated in vitamin A induced abnormal tail regenerates. Immune positive cells for both these enzymes are observed to be more in the treated tail regenerates. Besides, three fibroblast growth factors (FGFs) 1, 2 and 10 are expressed in the tail regenerates.

Introduction

Regeneration, the replacement of lost or damaged tissue(s) or organ(s) of an organism, is a wide spread phenomenon in animals. It can occur by an outgrowth of new tissue from the surface of the wound (epimorphosis) or by remodeling of the remaining parts (mophallaxis). The remarkable phenomenon of regeneration was first observed with the discovery that *Hydra*, a fresh water polyp, could generate a complete body from a small piece by Trembley (Trembley, 1744). Regeneration in vertebrates was reported in the year 1768 by Spallanzani based on the fact that a variety of amphibians could regenerate their legs and tails following amputation. Since Spallanzani's first scientific description of the phenomenon of limb regeneration (Dinsmore, 1991; Tsonis and Fox, 2009), numerous workers have investigated the regenerative capacity in different groups of animals. Among the tetrapods, the

amphibians exhibit the highest degree of regenerative ability. Urodele amphibians regenerate different body parts throughout their life where as in anurans, regenerating capacity is restricted to the larval period. Since the capacity to regenerate is lost on the onset of metamorphosis of the anuran tadpole, it is an ideal model to study the mechanism of loss of regeneration. Tadpoles also exhibit the phenomenon of homeotic transformation where tails are converted into hind limbs induced by vitamin A and its derivatives (Mohanty-Hejmadi et al., 1992; Maden, 1993; Muller et al., 1996). It is a process of transdifferentiation of one type of cell to another and regeneration studies

Tadpoles of *Megophrys* sp.
Photo Credit: Abhijit Das

Pravati K. Mahapatra

PG Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar-751 004, Odisha, India

Corresponding author e-mail: mahapatra.pk.zool@utkaluniversity.ac.in



in anuran tadpoles can give insight into the mechanism of such transformation of cells.

Role of extrinsic factors during regeneration

Regenerative processes in amphibians are known to be influenced by several extrinsic factors. It has been described that X-irradiation inhibits regeneration by preventing cell division (Maden and Wallace, 1976). Nerves have also been described to be essential for limb regeneration in all known species excepting the aneurogenic limbs regeneration of some larval amphibians (Kumar et al. 2011). Extracts from nervous tissues have been shown to have effects on blastema cell synthetic activity. Ultrasonication has shown inhibition of limb regeneration in urodele amphibians (Pizzarello et al. 1975). Actinomycin which inhibits transcription, when applied to the tail amputated tadpoles of *Ambystoma tigrinum* and *Rana pipiens*, greatly reduces the amount of regenerating tissue and at the same time increases the thickness of apical epidermal cap (AEC) which covers the wound after amputation and plays an important role in amphibian regeneration both in anuran and urodele amphibians (Wolsky, 1988). All the extrinsic factors without exception either had no effect or inhibited the process of regeneration. None showed any modulating

effect on pattern formation. These factors either permitted or inhibited regeneration but never altered regeneration in any way.

Influence of retinoids on pattern formation

The extrinsic factors that have been shown to directly influence pattern formation in amphibian tail and limb regeneration is vitamin A and its derivatives, the retinoids (Maden 1993; Ju and Kim 1994, 2010; Mahapatra, 1994). The retinoids include retinol, retinal and retinoic acid. The inhibitory and modifying influence of Vitamin A on tail regeneration in the anuran tadpoles of *Bufo andersonii* was reported for the first time by Niazi and Saxena (1968). Dose dependent inhibition of tail regeneration in two urodeles, i.e., *Notophthalmus viridescens*, *Ambystoma mexicanum* and one anuran *Xenopus laevis* following vitamin A (palmitate) treatment was reported by Scadding (1987). However, there was no evidence of any case of duplication of any part of the tail structure. The finding that vitamin A can induce homeotic transformation of tail to limbs in the marbled frog *Uperodon systoma* showed another remarkable effect of vitamin A (Mohanty-Hejmadi et al. 1992) i.e., transdifferentiation of cells of the tail into the cells of pelvic girdle and hindlimb.

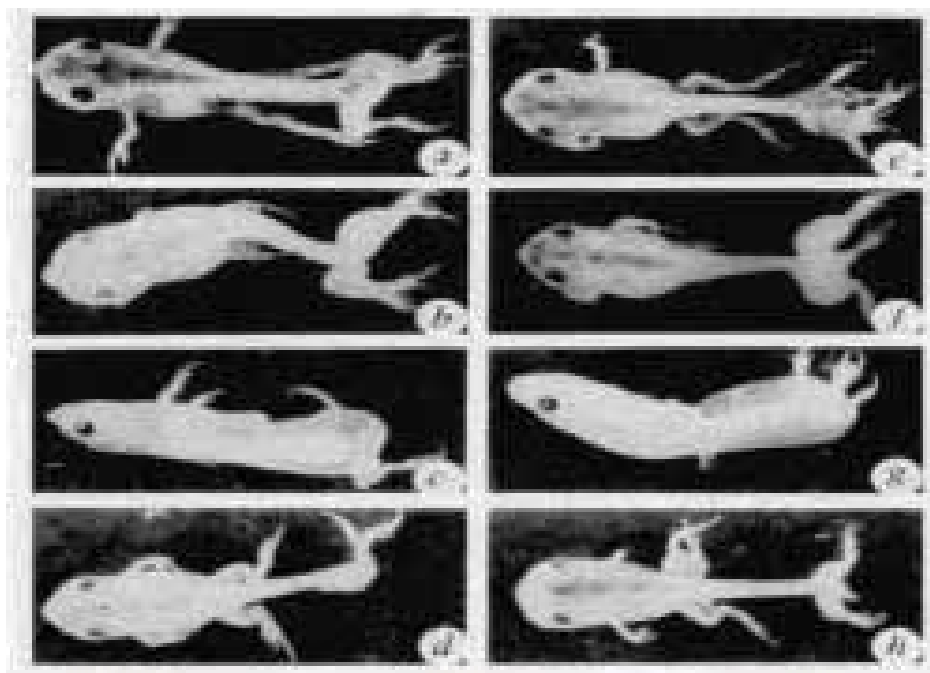


Figure 1: Homeotic Transformation of tails to limbs in the tadpoles of *Uperodon systoma*. (Source: Mohanty Hejmadi et al. 1992)

Following the initial finding, there were several reports on vitamin A induced inhibition of tail regeneration and homeotic transformation in different anuran species namely *Polypedates maculatus*, *Bufo melanostictus* (present name *Duttaphrynus melanostictus*), *Microhyla ornata* (Mahapatra, 1994; Mahapatra and Mohanty-Hejmadi, 1994, Mohanty-Hejmadi and Crawford, 2003) and *Rana tigerina* (Present name *Hoplobatrachus tigerinus*) (Das and Dutta, 1996). This phenomenon of homeotic transformation was also confirmed in two temperate anurans i.e., *R. temporaria* (Maden, 1993; Maden and Corcoran, 1996; Muller et al. 1994, 1996) and *R. ridibunda* (Muller et al. 1994, 1996). Maden (1993) reported induction of ectopic limbs by vitamin A 10IU/72 hours treatment upto the 54 stage (Nieuwkoop and Faber, 1967), which are comparable to Gosner (1960) stage 33-34 (limb paddle stage with toe

demarcation) tadpoles. In *R. temporaria* tadpoles, Muller et al., (1996) observed ectopic limb induction from stage 26 to 31 (limb bud to elongated buds with distal paddle-shaped structure) following 10IU/72 hour treatment of vitamin A palmitate. They also reported homeotic duplication of a whole body segment including vertebral elements, pelvic girdle elements and limb buds at the mid tail level.

Ectopic and normal hind limbs follow the same developmental pathway

Normal looking tails regenerated in the control tadpoles within 15 days of tail amputation while abnormal tails regenerated in the treated tadpoles in different anuran species. In more than 20% abnormal tails, bud like structures appeared which subsequently developed into ectopic hind limbs (Fig.2).

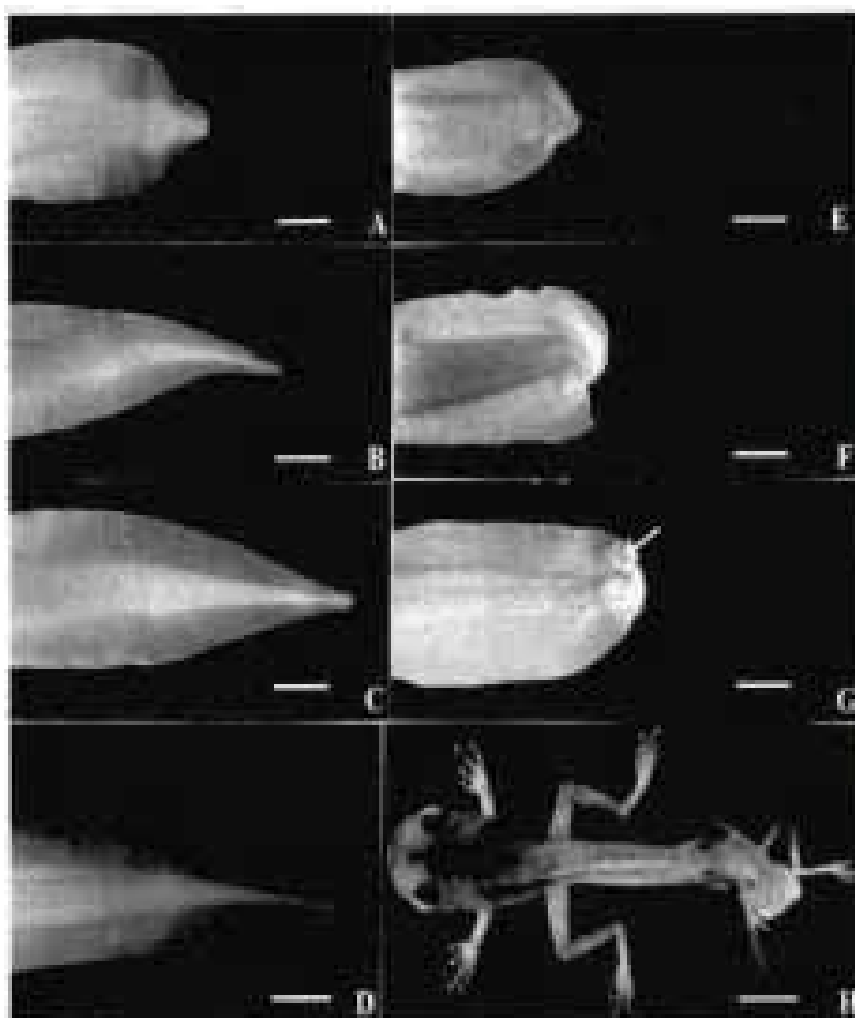


Figure 2: Morphology of the original and regenerated tails of the tadpoles of *Polypedates maculatus*. (A). Regenerated tail of the control group 5 days after amputation. (B). Tail of control group 10 days after amputation. (C). Normal looking tail of the control group 15 days after amputation. (D). Original tail before amputation. (E). Abnormal tail regeneration with a blunt end 5 days after amputation (F). Further enlargement of the abnormal tail 10 days post amputation (G). A bulbar mass in 15 days post amputated tail with limb buds (arrows). (H). A treated tadpole with ectopic limbs at the cut end of tail at the emergence of forelimbs (50 days post amputation). (Scale bar: Fig. A to G=2mm; Fig. H=5mm) (Source, Patnaik et al. 2012)



Marked histological similarities was reported to exist between normal and vitamin A induced ectopic limb buds in the tadpoles of the Indian tree frog, *Polypedates maculatus* (Mahapatra et al. 2004). However,

close association of nephric tubules and lateral plate mesoderm, as seen in normal hind limb bud did not seem to be essential for ectopic limb development (Figs.3 and 4)

Metamorph of *Rhacophorus* sp.
Photo Credit: Abhijit Das

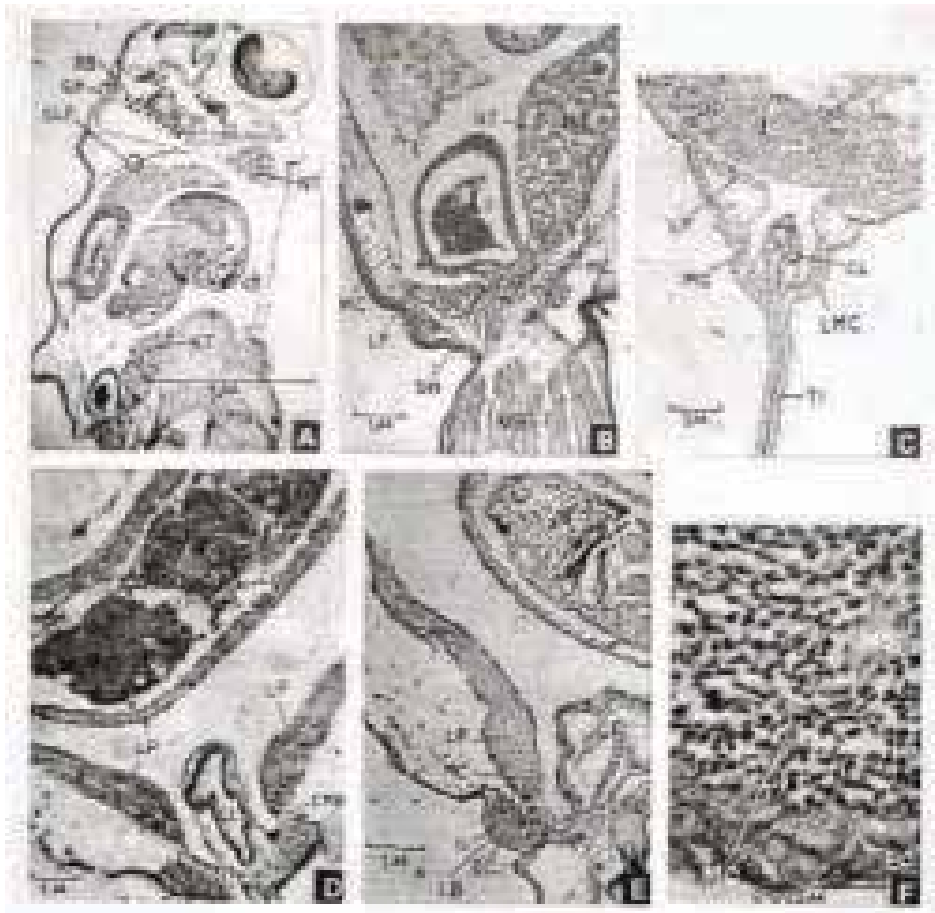


Figure 3: (A) L.S. of Gosner stage 24 showing the trunk with distinct branchial basket (BB), Kidney tubules (KT), muscle bundles (MB), somatopleure (SP) and splanchnopleure (SLP) (B) L.S. of Gosner stage 24, showing migratory mesodermal cells (MC) at the site of hindlimb bud formation (SH) and by the side of lateral plate (LP), nephric tubule (NT) in close association with mesodermal cells (MC). (C) L.S. of early feeding stage (Gosner stage 25), showing loose mesenchymal cells (LMC) at both sides of cloaca (CL) and prominent lateral mesodermal cells (MC). (D) L.S. of Gosner stage 26, showing the connection of mesodermal band (CMB) to the developing limb bud (LB). (E) L.S. of tadpole at Gosner stage 28 showing outer epidermal layer (EC), inner thickened mesodermal cells (MC) and the intervening layer (IL), (F) L.S. of Gosner stage 28, showing marginal sinus (MS) with in mesodermal cells (MC) and multilayered epidermal cells (EC). (Source: Mahapatra et al. 2004)

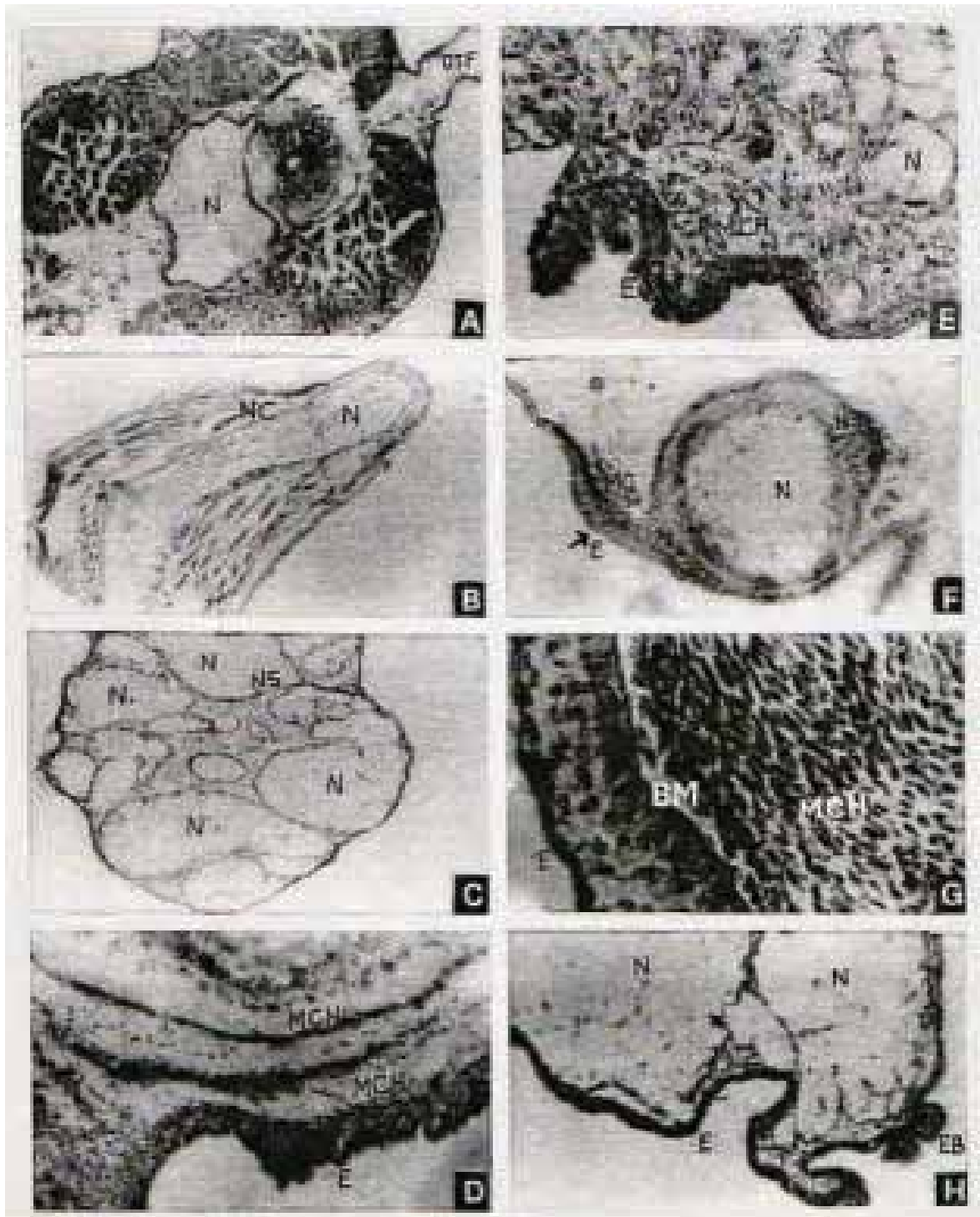


Figure 4: (A) T.S. through tail of a control tadpole, showing notochord (N) spinal cord (SC) and muscle bundle (MU). (B) L.S. through vitamin A treated regenerated tail, showing enlarged notochord (N) and small nerve cord (NC). (C) T.S. through tail, showing notochordal mass (N) in globules surrounded by notochordal sheath (NS). (D) L.S. through tail, showing folded epidermis (E) and two layers of condensed mesenchymal cells (MCH) below the epidermis. (E) L.S. through tail, showing thick epidermis (E), mesenchymal cells (MCH), compact and small globules of notochordal cells (N). (F) L.S. through abnormal tail, showing folded epidermal layer (E), large notochord (N) with thick notochordal sheath (NS) and accumulation of mesodermal cells (MC) beneath the ectoderm (Arrow). (G) L.S. through tail, showing thick epidermal layer (E), dark basement membrane (BM) and inner mesenchymal cells (MCH) with intercellular space. (H) L.S. through abnormal tail, showing vacuolated notochordal cells (N) and two ectopic limb buds (EB) protruding from the epidermal layer (E). (Source: Mahapatra et al. 2004)

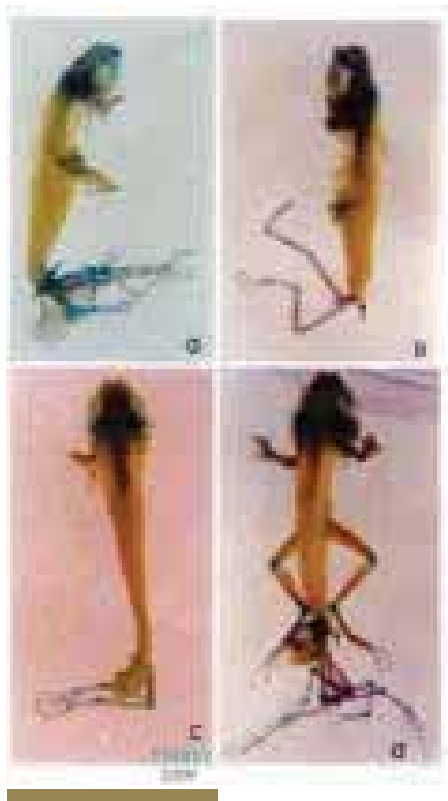


Figure 5: Fig.5 Ectopic hindlimbs showed both bony and cartilaginous elements (a–d). Pelvic girdles indicated as black arrows (a,b,d). (Source: Mahapatra, 1994)

Ectopic limbs were always hindlimbs with distinct thigh, shank, ankle and digits. Generally the ectopic hindlimbs were smaller than the normal hindlimbs and developed in pairs. In more than 50% cases

ectopic limbs originated from distinct pelvic girdles.

Role of oxidative stress during vitamin A induced abnormal tail regeneration

Oxidative stress state of the original, regenerating tails of the control and vitamin A treated groups were reported for the first time (Mahapatra et al. 2002) in the tadpoles of the Indian tree frog *Polypedates maculatus*. Lipid peroxidation (LPX) was investigated as an index of oxidative stress. Hydrogen peroxide (H₂O₂) was estimated to quantify level of this potent oxidant. Besides, two enzymatic antioxidants, i.e., superoxide dismutase (SOD) and catalase (CAT) related to normal development of anurans were estimated. The level of a non enzymatic antioxidant, reduced glutathione (GSH) normally expressed during cell division was also estimated. There was always a higher level of oxidative stress in the regenerating tails of the tadpoles. The level of oxidative stress further increased in the regenerated tails of the vitamin A treated tadpoles where there was abnormal tail regeneration. Thus, it was established that a hyper oxidative stress condition prevailed in the abnormal tails which is a pre requisite for ectopic limb development (Table 1).

Polypedates maculatus
Photo Credit: Abhijit Das



Table 1: Table1 Changes in oxidative stress parameters of the regenerated tail of control (C) and vitamin A treated (T) tadpoles of *Polypedates maculatus* (Mahapatra et al. 2002)

Oxidative stress parameters	Group	Days following tail amputation			
		5	10	15	20
LPX1	C	1.16*	1.08	1.8	0.63
	T	1.61	1.91	1.16	1.07
H2O22	C	1.87	1.88	1.82	1.14
	T	3.85	5.37	2.04	1.27
SOD3	C	14.37	8.1	6.9	0.91
	T	9.06	9.38	4.19	0.9
CAT4	C	1	1.21	1.3	1.09
	T	1.6	1.96	1.81	2.12
GSH5	C	2.11	1.4	1.15	1.15
	T	2.11	1.67	1.29	1.18

*Values in fold relative to original tail, C-control, T-treated group

- 1. Level of lipid peroxidation (nmol MDA formed/mg protein)
- 2. Level of H₂O₂ (nmol/mg protein)
- 3. Activity of SOD (units of SOD/mg protein)
- 4. Activity of Catalase (pmol/mg protein/min)
- 5. Level of Glutathione (μm/g tissue)

Role of phosphatases during vitamin A induced abnormal tail regeneration

Specific activities of acid and alkaline phosphatase

In the tadpoles of *Polypedates maculatus* and *Duttaphrynus melanostictus*, elevation of acid and alkaline phosphatase has been described during vitamin A induced abnormal tail regeneration, a pre requisite for ectopic organ formation (Patnaik et al. 2012; Mahapatra et al. 2015). Acid phosphatase is a lysosomal marker enzyme and is associated with lytic activities. As lysis of cell has been described during regeneration in anurans and urodeles (Carlson, 2005; Ju and Kim, 2010) this enzyme was estimated during tail regeneration. Alkaline phosphatase, a metalloenzyme attached to plasma membrane of cells (Moss, 1992) is a potent marker for undifferentiated stem cells (O'Connor et al. 2008; Keeling et al. 2009) and is also associated with regeneration in different groups of animals like planarians (Osborne and Miller, 1963) ascidian (Keeling et al. 2009), anurans (Junqueira, 1950) and

urodeles (Ghiretti, 1950; Karczmac and Berg, 1951; Schmidt and Weary, 1963; Inoue and Suzuki, 1969). As accumulation of undifferentiated cells takes place during tail regeneration (Mahapatra et al., 2004), activity of this enzyme was studied. Elevation in specific activity of acid phosphatase indicated lytic activities during regeneration and rise in specific activity of alkaline phosphatase was correlated with accumulation undifferentiated cells at the site of regeneration.

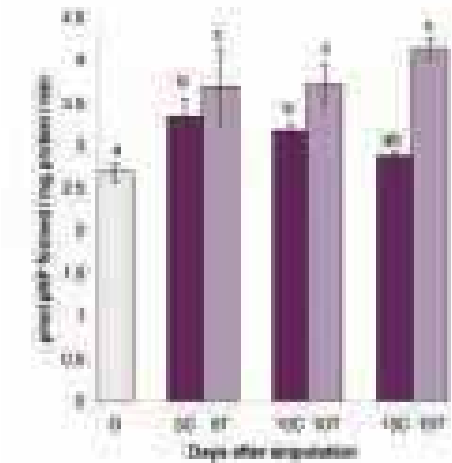


Figure 7: Specific activity of acid phosphatase in μmol p-nitrophenol (pNP) formed/mg protein/min at 37°C of the regenerated tails of vitamin A 10IU/ml treated (72h) and control tadpoles of *Polypedates maculatus*. O-original, C-Control, T-treated (Patnaik et al., 2012)

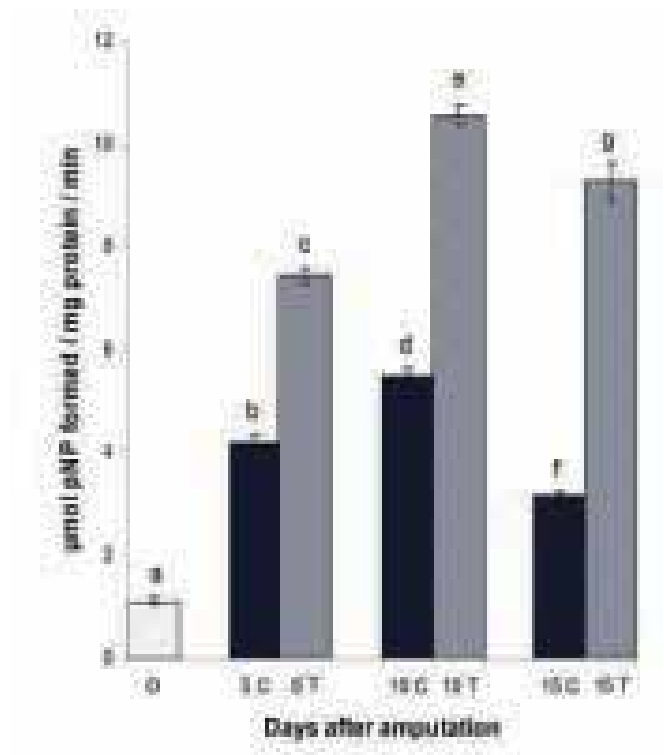


Figure 8: Specific activity of alkaline phosphatase in $\mu\text{mol p-nitrophenol (pNP) formed/mg protein/min}$ at 37°C of the regenerated tails of vitamin A 10IU/ml treated (72h) and control tadpoles of *Polypedates maculatus*. O-original, C-Control, T-treated (Patnaik et al., 2012)

Immunohistochemical localization of phosphatases

For immunohistochemical localization of acid and alkaline phosphatases, normal and regenerating tail tissues of control and vitamin A treated groups were considered. In the non-amputated and normally regenerated tails, acid phosphatase was majorly restricted to the epidermis and muscle patches although in the normally regenerated tails, notochordal sheath and spinal cord also stained for this enzyme. In vitamin A treated tails, acid phosphatase was mostly localized in the epidermis, notochord precursor cells and undifferentiated cells of the mesenchyme. Notochordal cells and notochordal sheath also showed positive staining (Fig. 9). Since, acid phosphatase was majorly expressed by tissue forming precursor cells, this enzyme has been suggested to be involved in tissue remodelling processes (Mahapatra et al., 2017).

Epidermis of the uncut tails showed no

immunoreactivity while the regenerating tails of both control and treated groups expressed alkaline phosphatase (ALP). In the treated tails, in addition to the epidermis, ALP was expressed in the layer below basement membrane, undifferentiated cells lodged in muscle and mesenchyme, spinal cord, notochordal sheath, notochord precursor cells and also blood vessels (Fig. 10). ALP positive cells were more in the treated tails than their corresponding controls. In treated groups, the distal portion of the tails showed higher ALP expression than proximal part. Such differential expression of ALP can be correlated with quantity of undifferentiated cells in the distal portion of the tail where the normal process of regeneration is interrupted and the tail regenerates abnormally, a pre requisite for ectopic organ formation (Unpublished data).

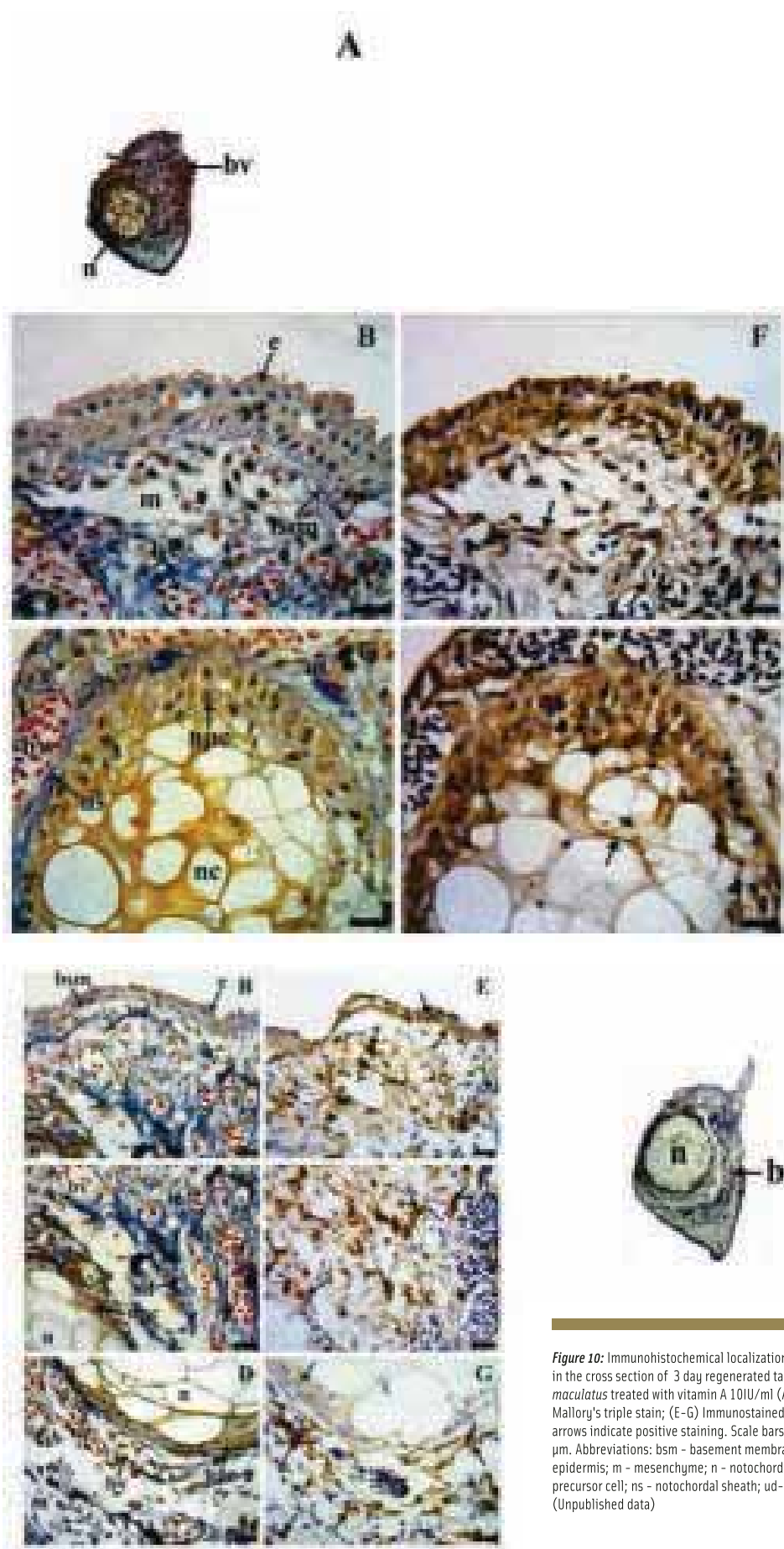


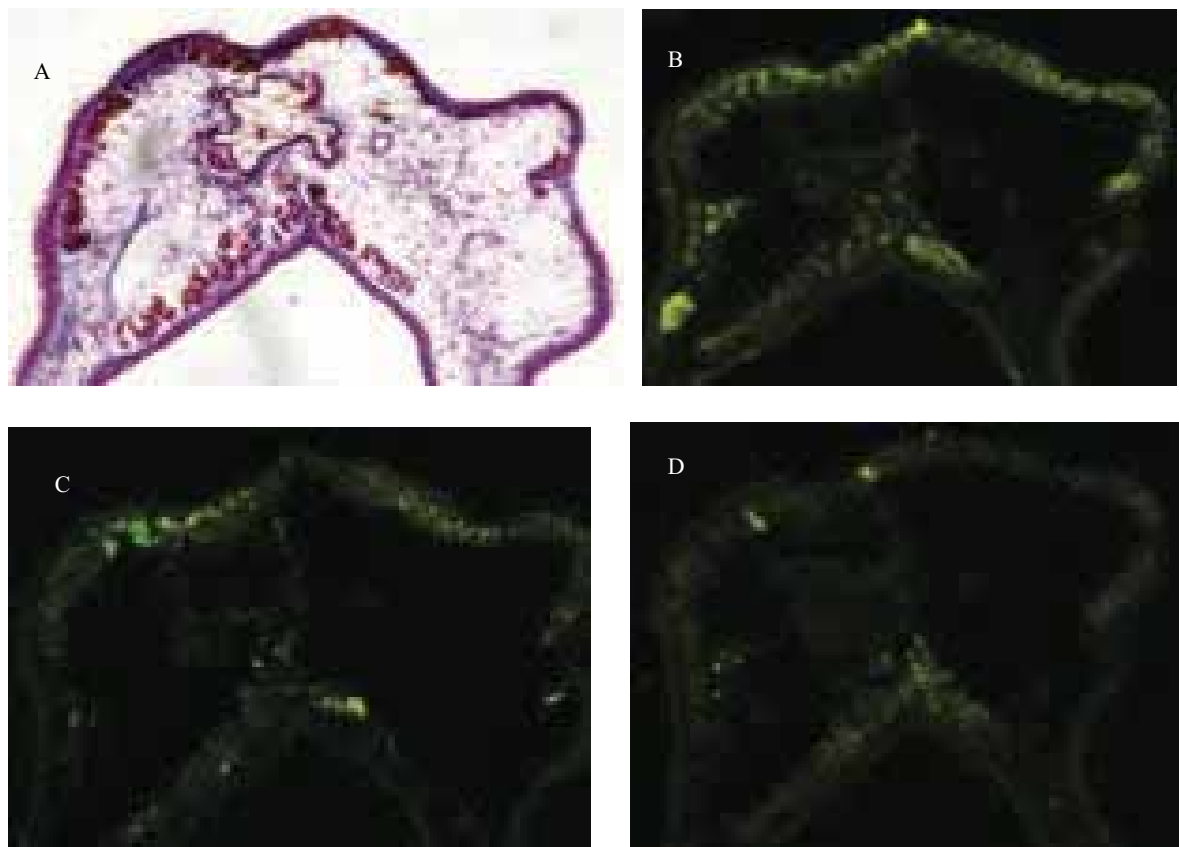
Figure 9: Immunohistochemical localization of acid phosphatase in the cross section of 3 day regenerated tail of *Polypedates maculatus* treated with vitamin A 10IU/ml (A-C) Sections stained with Mallory's triple stain; (D, E) Immunostained sections where black arrows indicate positive staining. Scale bar A=100 μ m, B-E= 20 μ m Abbreviations: apc - apoptotic cell; bsm - basement membrane; bv - blood vessel; e - epidermis; m - mesenchyme; n - notochord; nc - notochordal cell; npc - notochord precursor cell; ns - notochordal sheath (Source: Mahapatra et al. 2017)

Figure 10: Immunohistochemical localization of alkaline phosphatase in the cross section of 3 day regenerated tail of *Polypedates maculatus* treated with vitamin A 10IU/ml (A-D) Sections stained with Mallory's triple stain; (E-G) Immunostained sections where black arrows indicate positive staining. Scale bars: (A) = 100 μ m; (B-G) = 20 μ m. Abbreviations: bsm - basement membrane; bv - blood vessel; e - epidermis; m - mesenchyme; n - notochord; npc - notochord precursor cell; ns - notochordal sheath; ud - undifferentiated cell. (Unpublished data)

Role of fibroblast growth factors during retinoic acid induced tail regeneration

Fibroblast growth factors (FGFs) are well known for their diversified roles during proliferation and wound healing processes. These ubiquitously present growth factors are best known to operate across the epithelial- mesenchymal boundaries and such interactions have been reported to be

critical during appendage development. Interestingly, when reports of development of several ectopic limbs during tail regeneration in Vitamin A palmitate treated anuran tadpoles of *Uperodon systoma* came into limelight (Mohanty-Hejmadi et al. 1992), the regeneration research became more challenging and the role of FGFs in channelizing the epithelial-mesenchymal interactions have been emphasized in our present study.



In harmony with the earlier reports of the teratogenic effect of Vitamin A palmitate during tail regeneration, retinoic acid (RA) treatment during tail regeneration has also yield the obvious results. A model was proposed by Bryant and Gardiner (1992) to describe vitamin A induced transformation of tail to hind limbs along with pelvic girdle. According to this model, cells at the amputation site change their positional value to flank positional value due to vitamin A treatment. As a result of this change in positional value, from the cut end of tail hind limbs along with pelvic girdle developed instead of tail. FGF1 and FGF2

are potent neurotrophic factors and established mitogens and FGF10 has been known to be an important factor during limb bud initiation. Considering, the above potentialities of these growth factors, immunofluorescence localization was studied in the tissue sections of the regenerating tail and also in the retinoic acid treated regenerates of the tadpoles of *Polypedaus maculatus*.

In the retinoic acid treated tadpoles, stronger immunolocalization of FGF2 and 10 were noted in the tissue regenerates 72 hours onwards. However, FGF1

Figure 11: (A) Mallory staining of a retinoic acid treated abnormal tail section; (B, C, D) Immunofluorescence localization of FGF10, 2 and 1, respectively in retinoic acid treated abnormal tail. (Scale bar = 50 μ m). (Unpublished data)

immunoreactivity remained low and were mostly found in the regenerating spinal cord. Such cells were also found immunopositive for FGF2 suggesting their neurotrophic action. Since, the immunolocalization of these growth factors was more intense in the retinoic acid treated abnormal tails, their involvement during transdifferentiation of tail cells to limbs is suggested.

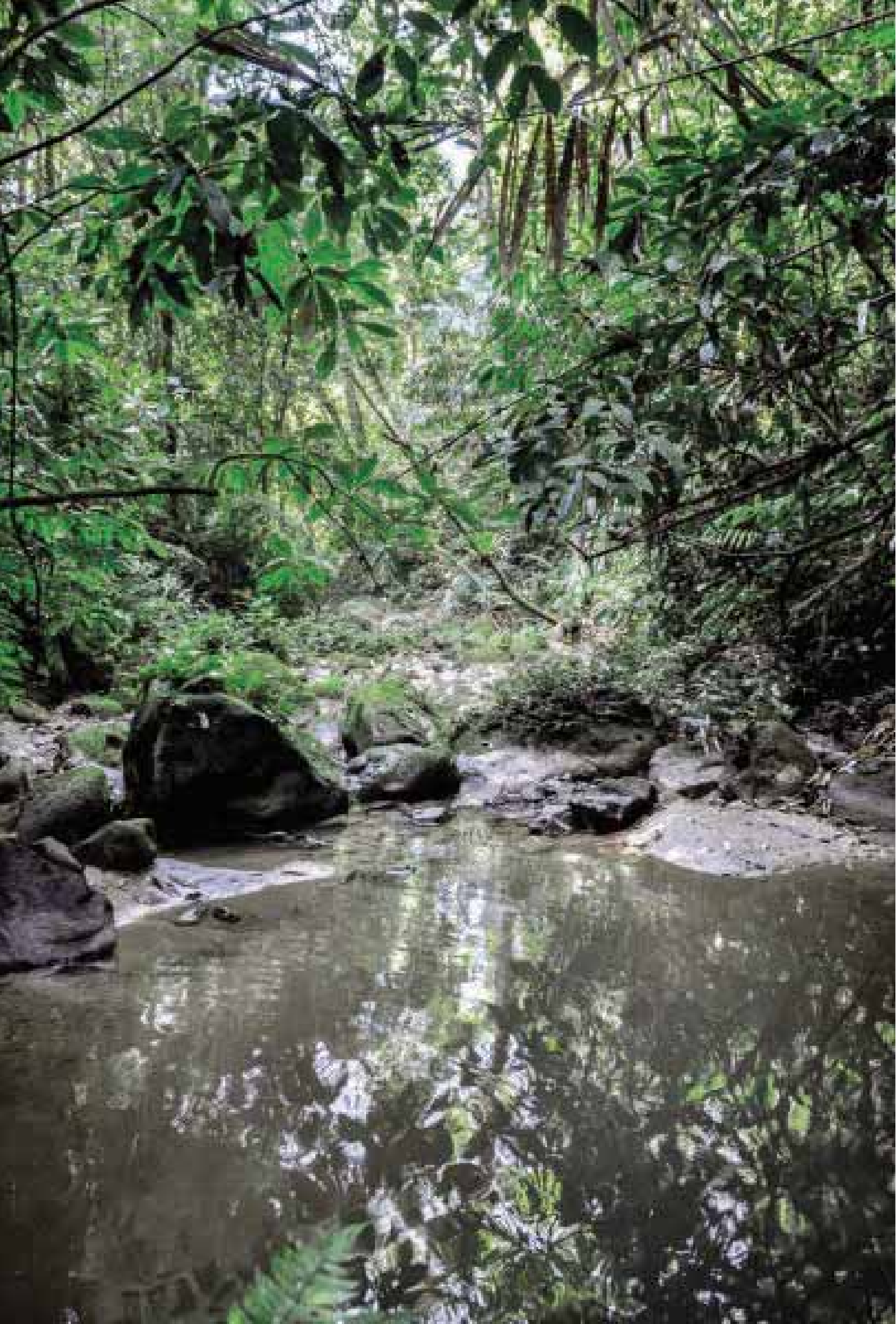
Conclusion

Frog tadpoles are easy to rear in laboratory conditions and are excellent model for regeneration studies of an organ. Since the regenerating capacity is lost soon after metamorphosis, there develops a block from regenerating to non-regenerating state. Understanding the mechanism that stops regeneration to occur soon after metamorphosis can unfold the mystery of loss of regenerating capacity in higher vertebrates.

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Biology of a Microhylid Frog, *Kaloula pulchra* Gray, 1831 (Amphibia: Microhylidae) in Mizoram, Northeast India

Abstract

The detailed biology of a microhylid frog, *Kaloula pulchra* was studied in natural and laboratory conditions. There was no detailed information on the ecology, breeding behaviour, development and feeding biology of this species before. This study provides the distribution, microhabitat ecology, breeding behaviour, development and feeding biology of *K. pulchra* in Mizoram. The present works revealed that *K. pulchra* was mostly found in the temporary waterlogged microhabitat in and around human habitation, starts to breed from late February i.e., the onset of monsoon to June, the advertisement call of male consisted of a single note emitted at an interval of 2.876 - 3.902 s (Mean \pm SD = 3.344 \pm 0.386, n = 10), number of eggs deposited by female ranges from 363 - 576 (N = 50) with a mean of 482.3 \pm 7.37 and no parental care was observed. It was found that the life cycle was completed within 47 \pm 2 days in both natural and laboratory conditions where water temperature ranged from 14.5°C - 29°C and 16°C – 28°C, respectively. It can be recommended that this species is an explosive breeder. Light and electron micrographs revealed that the oral apparatus at the larval stages i.e., tadpoles are devoid of jaw sheath and keratodonts. During a short period of metamorphosis, remodeling of the intestine of aquatic omnivorous tadpoles into terrestrial carnivorous adults was also observed.

Introduction

Common names for *Kaloula pulchra* Gray, 1831 include Beautiful Kaloula (Gray 1831), Asiatic Painted Frog (Karsen et al. 1986), Malayan Bullfrog, Ceylon Kaloula and Digging Frog (Ananjeva et al. 1988), Banded Bullfrog (Lim and Lim 1992; Chan-ard 2003; Grismer 2012), Malaysian Narrow mouth Toad (Frank and Ramus 1995), Painted Bullfrog (Das and Dutta 1998), Hainan Digging Frog and Piebald Digging Frog (Fei 1999), Painted Frog (Shrestha 2001; Dinesh et al. 2009), Painted Burrowing Frog (Nutphund 2001), Malaysian Bullfrog (Schleich et al. 2002), Painted Balloon Frog (Ahmed et al. 2009) and Painted Microhylid Frog (Mathew and Sen 2010). It is native to Asia like, Northeastern India (Meghalaya, Bihar, Assam, Nagaland, Tripura, Mizoram, and Manipur) east through Bangladesh and Myanmar and Thailand to southern China

(southern Yunnan, Guangxi, Hainan and Guangdong, and Taiwan), south to Singapore; Sumatra; Borneo; Sulawesi; introduced into Philippines (Luzon, Cebu, Marinduque, Mindanao, and Palawan) (Frost 2017). They occur naturally in a wide variety of habitats, from populated villages, to rice fields, streams at forest edge to leaf-covered forest floors. Like many other frogs, *Kaloula pulchra* lives in and around towns and avoids undisturbed forest areas. One of their characteristics is that they can burrow themselves into the soil with their hind

Key words:

Biology,
amphibian,
microhylid frog,
Kaloula pulchra,
Mizoram.

Kaloula pulchra.
Photo Credit: Abhijit Das

H.T. Lalremsanga

Department of Zoology, Mizoram University
Aizawl – 796004, Mizoram, India.

Corresponding author e-mail: htlrsl@yahoo.co.in



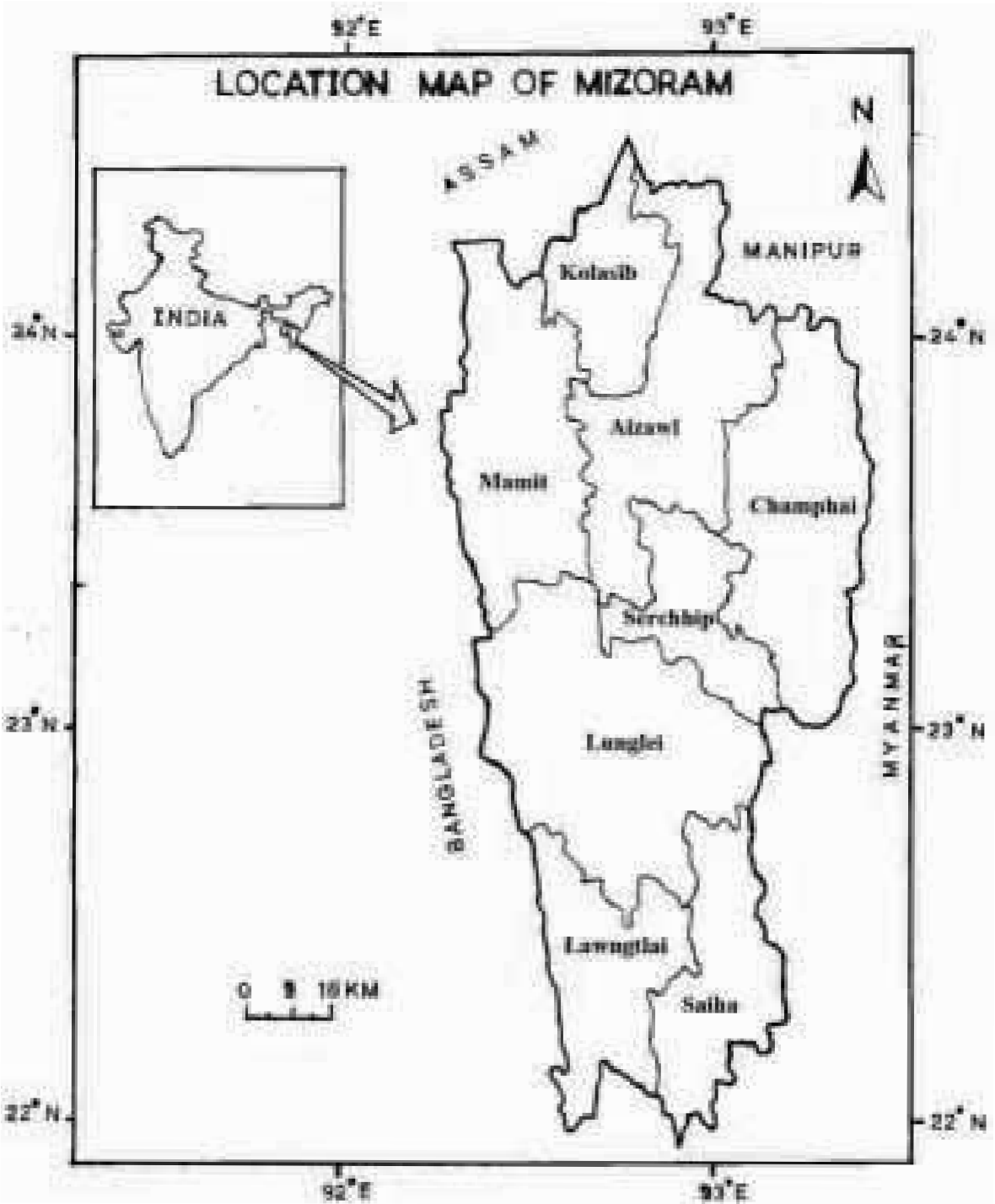


Figure 1: Location map of Mizoram

limbs quite efficiently. It is listed as Least Concern (LC) by IUCN version 3.1 in view of its wide distribution, tolerance of a broad range of habitats, presumed large population, and because it is unlikely to be

declining to qualify for listing in a more threatened category (Kuangyang et al. 2004). Chanda (2002) reported that although this species has been found in north east India, no data are available on its natural

history. This paper presents the ecology, breeding behaviour, development and feeding biology of *Kaloula pulchra* studied from Mizoram.

Methods

Distribution

To know the distribution of *Kaloula pulchra* in Mizoram, survey was conducted from the year 2004 and general observations of frogs were made throughout the forest, streams, rivers and other aquatic habitats, such as lakes, ponds, rice paddies, ditches and pools in plantations. The location (latitude/longitude) and elevation of the surveyed areas were determined with the help of Garmin (etrex) Global Positioning System (GPS) Standard survey techniques for amphibians include anuran calling surveys, egg mass surveys, larval surveys, and visual searches for adults. Specimens from different localities of Mizoram were collected. The surveyed areas represents all the eight districts, Aizawl district, Champhai district, Kolasib district, Lawngtlai district, Lunglei district, Mamit district, Saiha district and Serchhip district (Fig.1).

Sampling strategy

Extensive survey was conducted on egg masses, tadpoles and adults during day and night by Visual Encounter Surveys (VES) with the help of head lamps, torch light, bamboo torch and dip-net as well as mosquito net for larvae. Eggs and tadpoles collected from their natural environment were reared to metamorphosis for species confirmation by maintaining in plastic trays (38cm X 30cm X 8cm) containing stream or pond water along with algae for food in the laboratory condition.

Breeding season

Audio Encounter Surveys (AES) was used to identify locations where adult animals are attempting to breed. During the above survey period each selected sampling site was covered at different times of the day (from 2:00 PM to 11:00 AM on the following day) in order to record their breeding behaviours. Egg mass and larval surveys

provide evidence that mating occurred. The number of egg masses is also an indication of the number of adults that bred at that location.

Breeding behaviour, duration of amplexing and freshly spawned eggs were studied and documented with the help of photographic and video cameras. Rainfall data for the study sites were obtained from the Directorate of Agriculture & Minor Irrigation, Aizawl. The duration when the breeding pairs remained in amplexus was noted down and the temperatures of atmospheric as well as water and relative humidity of the study sites were recorded with hygrometer. Water pH was measured with the help of pH pen (S252873 HANNA Instrument) in the field.

The total numbers of eggs in the obtained clutches were counted and the morphometric measurements of amplexing pairs were measured.

Study sites

During the study period, it was observed that *Kaloula pulchra* was a seasonal breeder and its breeding activity coincides with the onset of monsoon i.e. late February to June in Mizoram. The study was conducted from 2004 to 2013 at two study sites, i.e., site-I: It includes rock-pools found on the bed of forest-edge intermittent stream at Sihhmui (23° 47.913'N -48.593' N and 92° 38.937' E- 39.203' E; 180m-184m asl.), Aizawl district. These temporary rock-pools are completely or incompletely isolated from the main stream which is one of the tributaries of Tlawng river. The stream bed is rocky, intermittent, shallow or temporarily deep in some areas. The circumferences of the pools range from 97 cm to 325 cm with about 10 cm to 45 cm in depth, bottom contained a thin sandy soil with fallen leaves and twigs forming shelters for tadpoles (Fig. 2 a & b); site - II: It consists of temporary pools and small rock-pools surrounded by secondary forested area (23° 44.144' N and 92° 40.282' E; 865m asl) inside the campus of Mizoram University, Tanhril, Aizawl. The pools are about 250 cm - 360 cm in diameter, whereas those rock-pools measured about 30 cm - 75 cm in diameter (Fig. 3 a & b). The bottoms of all the pools were filled with leaf litters and humus that provided shelters for tadpoles.

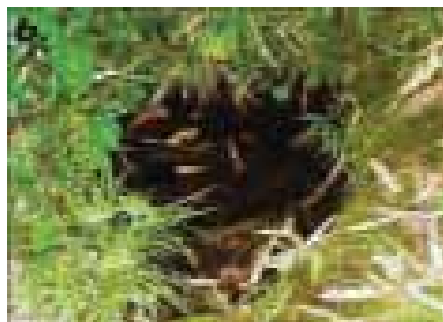


Figure 2(a & b) : Rock-pools in the breeding sites of *Kaloula pulchra* at Study site-I

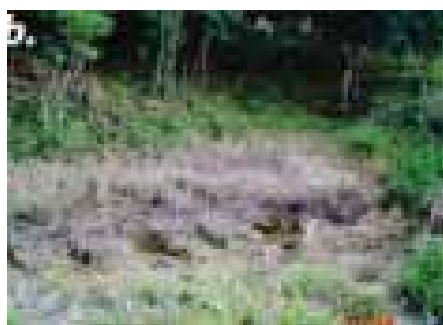
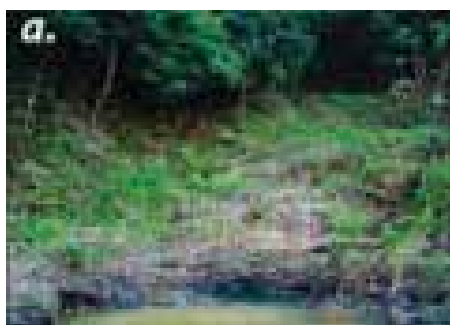


Figure 3(a & b) : Temporary pools, the breeding sites of *Kaloula pulchra* at Study site-II

Acoustic analysis

Mating calls were recorded with the help of digital voice recorder Samsung SVR 380 (FM frequency range 87.5-108 MHz). The sampling rate used to convert the signals to digital format was 8 KHz with 16-bit precision. The oscillogram was prepared and analyzed with the help of a software tool "SoundRuler Version 0.9.6.0 (acoustic analysis)". The notes are composed of groups of pulses. Notes are measured from the beginning of the first pulse to the end of the last pulse; intervals between two subsequent notes are measured from the end of the last pulse of the first note to the beginning of the first pulse of the following note; note repetition rate is the number of notes per second; pulse repetition rate is the number of pulses per second.

Development and metamorphosis

Amplecting pairs from the above study sites were collected and brought to the laboratory and allowed to lay their eggs in the laboratory and were maintained in a plastic tray (38cm X 30cm X 8cm) containing water, algae and debris collected from the study sites to allow further development and metamorphosis. During the study period, the freshly spawned eggs were also collected after they were laid at the study

sites and brought to the laboratory for further studies. Some egg masses were fixed immediately in the field in a mixture of 5 % formaldehyde and 70% ethanol in a ratio of 1:1, and after reaching the laboratory, the eggs were separated from the masses and counted to know the clutch size.

Temperature and pH of water was maintained as in the natural condition and the water was changed every alternate day. The rate of development was observed under a stereoscopic dissecting binocular microscope (Labomed CSM2). The time of onset of each new stage was noted and some developmental stages were fixed in a mixture of 70% alcohol and 4% formaldehyde in the ratio of 1:1. Staging of the anuran embryos and larvae of both the species was carried out on the basis of a new external morphological change as per the criteria described by Gosner (1960). For measuring developing embryos and tadpoles, stage occulo micrometer and dial vernier caliper (Mitutoyo series No. 505-671) accurate to 0.02 mm were used, respectively. Photographs of the developmental stages were taken with the help of microscope (Labomed CSM2) with photographic attachments.

The hatched tadpoles were reared in a plastic tray containing pond water collected

from the study sites and fed daily with algae gathered from the breeding sites. The temperature in the laboratory conditions was monitored. In order to know the stages attained by the developing tadpoles, regular observation was conducted depending on the rate of development. Simultaneous observation was conducted both in the natural and laboratory conditions.

Food and feeding behaviour in relation to oral structures and intestines

After collection in the field, the tadpoles were immediately fixed in 4% formaldehyde and autopsied for analysis of gut content. The foregut of a tadpole close to the oesophagus was cut and its contents teased on to a glass slide or petridish. Gut wall and visible portions of lining were removed with several drops of distilled water. The gut contents were spread as thinly as possible; a cover slip placed over it and was observed under a compound microscope. Identification on the food items of the tadpoles was made following the method of Turner (1892), Edmonson (1959), Needham and Needham (1972), and Fritsch (1979). The feeding habits of adults was studied by removing the stomach contents with the help of 10 ml Syringe connected with rubber tube and analyzed under a stereoscopic microscope.

The oral structures of the selected developing tadpoles were studied using

light microscopy, stereoscopic binocular microscope following the criteria of Altig and Mc Diarmid (1999), and also with Scanning Electron Microscopy.

For scanning electron microscopy, the samples were washed with double distilled water, fixed in 2.5% Glutaraldehyde solution (Prepared in 0.1M Na-Cacodylate buffer) for 4 hours and post-fixed in 1% Osmium tetroxide buffered (0.1M Na-Cacodylate buffer) for 1 hour at 4°C. The pH of the fixative and buffer was maintained at 7.4. The samples were then dehydrated through ascending acetone grades and drying was done in Tetra methyl silane (Dey et al. 1989). A thin conductive coating of gold was applied to the samples using a JFC 1100 (Jeol) ion sputter and the coated samples were examined with the aid of a JSM-35 CF (Jeol) scanning electron microscope at an accelerating voltage of 20KV. Working distance (WD) was at 15mm and the necessary tilting angles were used to view the frontal portion of the oral structures.

Histology

Histology of the gonads of both adults and the developing gut walls of tadpoles will be studied by using the method given by Rugh (1962).

Data Analysis

The data were analyzed statistically with the help of statistical software tools SPSS (7.5.1 version) and OriginPro 8 SRO (8.0724 version).

Uperodon taprobanica is widely distributed in India and also known from Western Assam. Photo Credit: Abhijit Das



Results

From the present investigation, the biology of a microhylid frog, *Kaloula pulchra* in Mizoram was documented:

Distribution and microhabitat

In the present survey, *Kaloula pulchra* was encountered naturally in a wide variety of habitats such as in and around human habitations, cemented tanks, swampy or temporary waterlogged areas, tree trunks, rock-pools and along forest-edge streams. It was found to be a nocturnal fossorial frog. They hide under leaf litter during the day hours and used to come out in the evening. For much of the time, the frogs stay out of sight by digging backward with their hind limbs into underground burrows, into piles of trash, and into other secretive spots they find along the ground. An individual was collected from a tree-hole of about 1.5 m from the ground (824 m asl) just outside Mizoram University main gate. Breeding

mainly takes place in temporary pools. During breeding season, they were collected mostly from rain-fed pools, water holes, permanent ponds, streams and puddles surrounded by vegetation or forested area and cemented tanks in residential areas. The collection sites as shown on the Table 1 ranged from lower altitude to mid-altitude between 180 m - 963 m asl.

Description and morphometric measurement of the specimens

Diagnosis: A stout-bodied, brightly-colored frog with short limbs and pointed head (Fig. 4). A dark triangular spot occupying the whole back from the middle of the eyelids and a lateral streak of the same color from the posterior corner of the eye, the two being separated by a yellow dorso-lateral stripe. Lower surfaces dark brown, mottled with light grey and cream colours; throat of the male infusate.



Figure 4: Male and female *Kaloula pulchra*

Description: Snout-vent length (SVL) of males ranges from 57.74 mm - 69.76 mm (N = 50) and females 59.90 mm - 69.94 mm (N = 50). Head broader than long; snout short, round; canthus rostralis rounded; loreal region oblique; interorbital space $1\frac{1}{2}$ to twice as broad as the upper eyelid; tympanum hidden. Vomerine teeth are absent (Fig. 5).

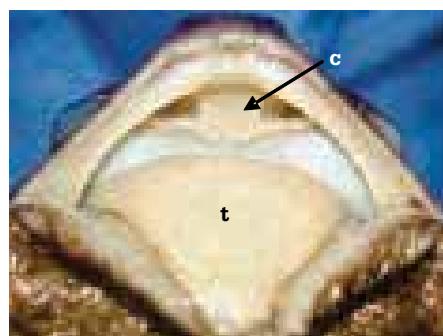


Figure 5: Mouth of *Kaloula pulchra*. t: tongue; c: chaonae.

Fingers with small truncate dilatations distally; relative length of fingers $F1 < F2 < F4 < F3$, the third finger longest and much longer than snout (Fig. 6a). Hindlimb rather short, mottled with brown, light grey and cream colours; toes short, scarcely dilated; relative toe length $T1 < T2 < T5 < T3 < T4$ with a rudiment of web (toe web formula $I1-1II\frac{1}{2}-2III1\frac{1}{2}-3IV3-1V$);



subarticular well developed (Fig. 6b); tibio-tarsal articulation reaching the shoulder; heels do not overlap when hindlimbs folded at right angle to the body. Both inner and outer metatarsal tubercles present. Skin smooth or with irregular flat warts above; an indefinite fold from the eye to the forearm and sometimes one across the occiput. Lower surfaces smooth or faintly granular.



Figure 6 a & b: Left hand and left foot of *Kaloula pulchra*

Secondary Sexual dimorphism: Males have darker throats than females. Females are generally larger than males.

Tadpole description: (see Fig. 15.26a&b; Fig. 15.30a&b; Fig. 15.34a&b)

Diagnosis: Body light to dark-brown with scattered black spots up to the level between eyes, tail muscle mostly black with small, creamy streak, tail fins more or less transparent with dark patches.

Morphology: Body oval, snout blunted, depressed; eyes dorso-lateral, nostrils dorsal, not open, equidistant between snout tip than eye; spiracle posterior mid-ventral, extended as a short tapered tube, inner wall free from body, spiracular opening directed postero-ventrally; medial vent tube continuous with ventral fin. Tail lanceolate, dorsal fin margin weakly convex, both margins tapering abruptly at distal towards a narrowly pointed tip. Dorsal fin originating at body-tail junction, slightly deeper than

ventral. Naso-lacrymal grooves present.

Colour/Markings: Dorsum and flanks light to dark-brown with scattered black spots, tail muscle mostly black with small streak, and tail fins creamy with dark patches; venters pigmented with dark patches.

Oral Disc: Mouth terminal with dorsal and ventral semicircular labial flaps, no marginal papillae; both jaw sheaths and keratinized tooth row absent and no LTRF (Labial Teeth Row Formula).

Courtship, mating calls and spawning

In the present observation, the breeding activity starts from late February. Males of *K. pulchra* start calling from Late February, during both day and night hours. No calling activities were recorded after June though individuals (both males and females) were

encountered in the habitat. With the first shower frogs come out from their hibernation and started to call. During the early part of February, the atmospheric temperature ranged between 14°C to 28.5°C and relative humidity 42% - 86%. Within 2 - 3 weeks of the first shower when sufficient water was available for breeding activity in the dried breeding grounds (temporary ponds and rock-pools), male frogs start calling from the pool. Atmospheric

temperature ranged between 14°C to 33°C, rainfall between 3 mm - 539.7 mm and relative humidity fluctuates between 47% - 92%, water temperature 14.5°C to 28.5°C , pH between 6.2 - 6.7, during the study period in both the study sites. At the onset of dusk, males first enter the breeding site and float in the pools, and blow up their bodies to make calls (Fig. 7a&b).



Figure 7 a & b: Male *Kaloula pulchra* calling from the breeding ground

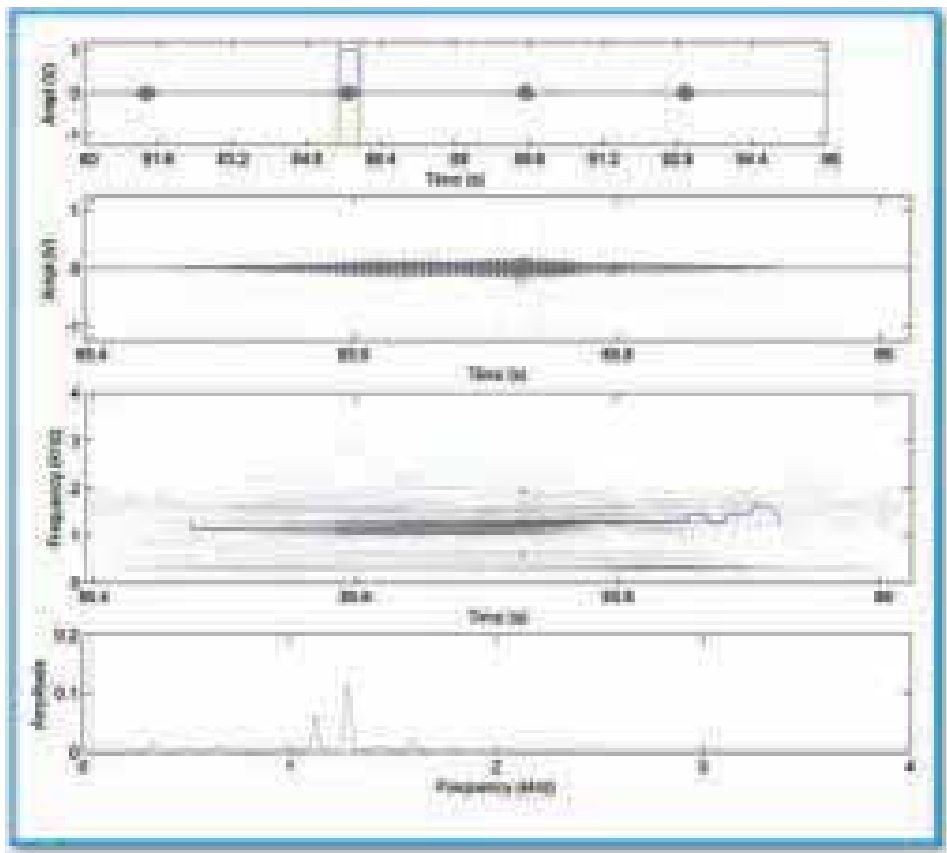


Figure 8 : Oscillogram, sonogram and frequency spectrum of an advertisement call of *Kaloula pulchra*

Calls are loud and deep like a bull moo. Advertisement calls were emitted in series with variable call intervals. The call consisted of a single note (Fig. 8) emitted at

an interval of 2.876 - 3.902 s (Mean \pm SD = 3.344 ± 0.386 , n = 10), the note repetition rate ranged from 0.21 - 0.25 notes per second (Mean \pm SD = 0.23 ± 0.015 , n = 10).

The notes lasted 450.8-620.3 ms (Mean \pm SD = 527.8 \pm 65.671, n=10) and were composed of a series of 36 - 45 pulses. The amplitude of the note increased quickly in

its second third, then decreased more slowly until the end, as did the amplitude of the pulses. The frequency spectra had a dominant band at 1265 Hz.

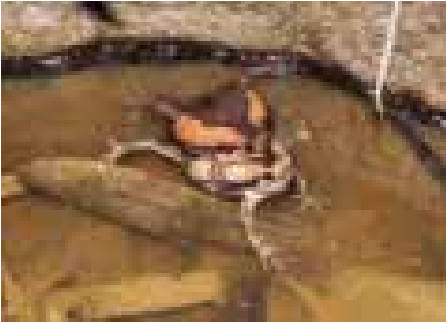
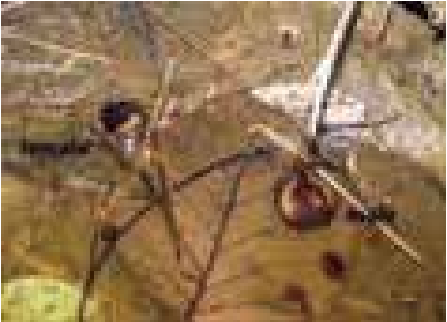


Figure 9 a & b: Mating behavior of *Kaloula pulchra* (a.) Female enters the breeding ground and approaching the calling male; (b.) Male immediately grabs the female from her back

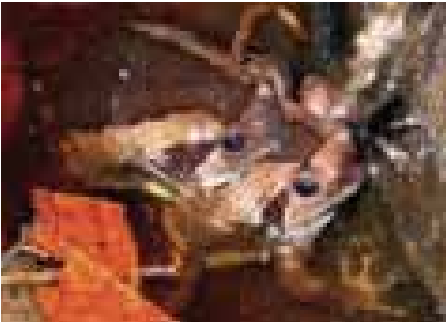


Figure 10 a & b : Axillary amplexing in *Kaloula pulchra*



Figure 11 : *Kaloula pulchra* amplexes with the female while other male continues to call

Male mating call started from around 4:00 PM and a single male vocalization can be heard from a distance of about 100 m away. In this observation, the distance between two adjacent calling males in the breeding ground are usually 2 m - 10 m apart. A single male usually occupied one rock-pool, if more than one male occupied a small rock-pool, the dominant male would drive out other males. In the present observation,

the existence of territorial combat for the breeding ground among males *Kaloula pulchra* was documented. Callings was in chorus, however adjacent calling males alternated their calls. In this study vocalization of unsuccessful male was noticed till 6:00 AM in the next morning. During observation in the fields, females were encountered in the forest-edge approaching the breeding ground in

response to the calls. Normally, after 2 - 5 hrs of continuous calling, females arrived in the breeding ground and mating was usually observed from 18:00 PM to 12:00 AM. After entering the breeding ground, the female approached the calling male from the back and with the help of her forelimbs she started tackling hind-limbs and flanks of the calling male. Suddenly, the male turned back and mounted on the back of the female (Fig. 9a & b). Pairs in amplexus can be seen in water submerging together usually in the corner of pool (Fig. 10a&b). Amplecting was axillary and a single pair was usually found to occupy each water-hole, where as two to three pairs were also encountered in the larger pools. In case of large breeding ground, a dominant male first mate with a female that first entered the pool while other males continued calling for other females (Fig. 11). Male-male combat for the female or sexual conflicts between male and female was not observed. Amplecting usually last for 20

minutes to 1 hr. Females laid eggs quickly which last for 10 minutes to 15 minutes.

After laying eggs, the female was seen leaving the breeding site, while the male was found to stay for a longer period of time around the clutch of pigmented eggs ($1.48 \text{ mm} \pm 0.01$ in diameter) floating on the surface of water (Fig. 12). After some time the male also left the eggs to hatch on their own (Fig. 13). Eggs were laid on the water surface mixed with leave debris (Fig. 14a & b). No parental care was documented in this species.

ANOVA tests on the difference of significant (at 0.05 level) between the breeding season and environmental factors (i.e. air temperature, relative humidity, rainfall, water temperature and pH of water) at study site-I were $p=0.604$, $p=0.458$, $p=0.375$, $p=0.087$ and $p=0.139$, while at study site-II, $p=0.598$, $p=0.094$, $p=0.603$, $p=0.828$ and $p=0.121$, respectively (Table 2 & 3).



Figure 12: Amplecting pair of *Kaloula pulchra*, after laying eggs.

Figure 13: After laying eggs, female *Kaloula pulchra* left the breeding ground, later followed by male.



Figure 14 a & b: Eggs were laid on the water surface mixed with leave debris

Size of amplecting pairs and clutch size

Kaloula pulchra exhibited a distinct sexual size dimorphism where males are with darker throat and smaller ($\text{SVL} = 61.92 \pm 0.49 \text{ mm}$; $N = 50$) than the females

($\text{SVL} = 64.60 \pm 0.51 \text{ mm}$; $N = 50$).

The clutch size varied from 363 - 576 ($N = 50$) with a mean of 482.3 ± 7.37 in different clutches. No correlation was found between the snout-vent length of females and clutch sizes ($r = 0.187$; $p = 0.167$). After taking the morphometric measurements of

adults and tadpoles, some were preserved and the rests were released back to their natural environment.

Weight of the testis of breeding males varied between 0.045gm - 0.087gm and that of ovaries ranged between 1.676gm - 2.531gm during the breeding period.

Development and Metamorphosis

Studies on the successive ontogenic changes during developmental process are important in understanding the ecology of species and for planning conservation measures. Appropriate staging of the larval period is, therefore, fundamental to various life history studies of amphibians.

During the course of this study the developmental stages were recorded from the time of egg laying till the embryo hatched into a tadpole, and metamorphosis of the tadpole into a froglet. The stages in the entire developmental series are selected on the basis of external morphological characteristics as described by Gosner (1960), and altogether 46 different developmental stages were recorded for each species. The measurements made in different stages include diameters of the eggs, sizes of early embryos and total lengths of larvae. For each stage, ten numbers were used for morphometric measurement.

Developmental Stages and Metamorphosis of *Kaloula pulchra*

Freshly spawned eggs masses were collected from the field and observed in the laboratory with water temperature ranged from 16°C - 28°C. The time of egg laying till the completion of metamorphosis was also monitored in the natural environment at the study sites I (water temperature 15°C - 28°C; pH= 6.0 - 6.7) and II (water temperature 14.5°C - 29°C; pH= 6.1 - 6.7). The life cycle of this species lasted for a very short period which is only about 47 ± 2 days (Table 4). A brief account of various stages of development and metamorphosis of *Kaloula pulchra* is given in the following and in the figures (15.1-46).

Stage 1 -Unfertilized egg: The newly laid egg is spherical in shape with the animal pole pigmented dark brown which slowly

fainted towards the creamy vegetal hemisphere. It measures about 1.48 ± 0.43 mm in diameter. It is enveloped by a thin, transparent, vitelline membrane. Around the egg is the jelly capsule (Fig.15.1).

Stage 2 -Fertilized egg: Within 25 minutes, a milky pigmented zone, the gray crescent starts to appear on the animal pole which indicates the penetration of male sperm inside the female egg. It measures about 1.48 ± 0.71 mm. This stage is observed within 25 mins (Fig.15.2).

Stage 3 -Two cell stage: A wide furrow appears in the animal hemisphere after fertilization which extends down through the gray crescent, continued towards the vegetal hemisphere, dividing the egg into two blastomeres. The embryo measures about 1.51 ± 0.64 mm. The first cleavage was completed in 45 mins after fertilization (Fig.15.3).

Stage 4 -Four cell stage: The second cleavage was meridional and right angle to the first cleavage, and it started from the animal. The complete four cell stage with four blastomeres was observed at 1 hrs 05 mins after fertilization. The embryo measures 1.52 ± 0.38 mm (Fig.15.4).

Stage 5 -Eight cell stage: The third cleavage is horizontal, latitudinal towards the animal pole and at right angle to the earlier cleavages. Eight blastomeres have formed. The micromeres of the animal pole are pigmented and the macromeres of the vegetal pole are slightly pigmented at the upper region and slowly unpigment towards the lower region. It was observed at 1 hrs 55 mins. The size measured 1.50 ± 0.27 mm (Fig.15.5).

Stage 6 -Sixteen cell stage: The fourth cleavage furrows are found to be vertical and formed sixteen blastomeres by division of each blastomere. The egg size measured 1.51 ± 0.53 mm. It was observed after 2 hrs 50 mins (Fig.15.6).

Stage 7 -Thirty two cell stage: The fifth cleavage is horizontal and cut each blastomere completely resulting in the formation of sixteen smaller micromeres and sixteen larger macromeres. It was recorded at took about 5 hrs and measures 1.52 ± 0.84 mm (Fig.15.7).

Stage 8 -Mid cleavage: The morula stage is characterized by the division of earlier stage. Pigmentation on the animal pole became a little bit darker and divided equally and smaller in size, while the unpigmented vegetal pole macromeres divided unequally. The size was increased and measured about 1.53 ± 0.52 mm. in diameter. It indicated the beginning of blastulation and was observed after 6 hrs and 55 mins (Fig. 15.8).

Stage 9 -Late cleavage: Blastulation was completed and the embryo looks granular. It took about 8 hrs and 20 mins and the size was increased and measured 1.55 ± 0.27 mm (Fig. 15.9).

Stage 10 -Dorsal lip: At this stage, the dorsal lip of blastopore was observed below the equator due to invagination of the micromeres at 9 hrs and 30 mins where the embryo measured 1.55 ± 0.63 mm (Fig. 15.10).

Stage 11 -Mid gastrula: Due to the continuous epibolic migration of micromeres over the vegetal hemisphere, the exposed area of macromeres was greatly

reduced and formed the yolk plug stage. It took about 11 hrs 15 mins and the gastrula measured 1.59 ± 1.45 mm (Fig. 15.11).

Stage 12 -Late gastrula: Due to continuous invagination of the micromeres the blastopore was gradually reduced at about 12 hrs and 45 mins and the slightly elongated late gastrula which measured 1.67 ± 1.29 mm in diameter was formed (Fig. 15.12).

Stage 13 -Neural plate: The embryo continued to elongate and the flattened dorsal surface formed the neural plate, and the elevated lateral ridges formed neural folds. The embryo measured 1.84 ± 0.75 mm and this stage was observed at 14 hrs and 55 mins (Fig. 15.13).

Stage 14 -Neural fold: The elongating embryo result in the formation of the neural fold at around 16 hrs and 40 mins and the it measured 2.39 ± 0.63 mm (Fig. 15.14).

Stage 15 -Rotation: Each neural fold growth towards one another and the neural groove became very narrow. The embryo continued to elongate antero-posteriorly. It

Kaloula assamensis is known from north bank of river Brahmaputra and northern west Bengal.
Photo Credit: M. Firoz Ahmed



was completed at 18 hrs and 05 mins and measured 2.40 ± 1.19 mm (Fig.15.15).

Stage 16 -Neural tube: The embryo was further elongated and a dorsal ridge was formed due to the fusion of the two neural folds. The neural tube measured 2.47 ± 0.42 mm and it was observed at 18 hrs and 45 mins (Fig.15.16).

Stage 17 -Tail bud: It was characterized by the protrusion at the posterior end of the embryo which became slightly curved toward the left side. Gill buds persisted in the cephalic region and this stage was observed at 19 hrs 15 mins. It measured 3.01 ± 0.74 mm (Fig.15.17).

Stage 18 -Muscular response: The tail buds elongated and became longer than wide and dark pigmentation could be seen at positions of future nares. The embryo started to hatch at this stage. It was observed at 20 hrs 10 mins and the embryo measured 3.57 ± 0.92 mm (Fig.15.18).

Stage 19 -Heart beat: Both dorsal and ventral portions of tail fin slightly developed and this stage was indicated by the pulsating heart. Rudimentary external gills slightly protruded and tail continued elongation. Heart beat was observed at 22 hrs 15 mins and the embryo measured 3.90 ± 1.23 mm (Fig.15.19).

Stage 20 -Gill circulation: Gills were elongated and oral sucker became well developed. Mouth started to open and cornea becoming prominent. The larvae started to swim. The embryo measured 4.33 ± 1.62 mm and it was observed at 1 day and 5:30 hrs (Fig.15.20).

Stage 21 -Cornea transparent: After 1 day and 11 hrs the cornea was transparent. The external gills attained their maximum length and the developing operculum covered the basal portions. Tail became straight and elongated. Oral suckers and nasal pits became prominent. The embryo measured 5.63 ± 1.35 mm (Fig.15.21).

Stage 22 -Tail fins transparent: Dorsal and ventral tail fins become transparent, and the head and trunk are distinctly demarcated. The oral suckers and the cornea were prominent. This stage was observed after 1 day and 21 hrs and it measured 5.93 ± 1.02 mm (Fig.15.22).

Stage 23 -Operculum present: After 2 days and 12 hrs, the operculum was well developed, and gills length shortened. The larva was elongated and measured 6.37 ± 1.28 mm (Fig.15.23).

Stage 24 -Operculum closes on right side: After 2 days and 23 hrs, it was observed that right external gill was disappeared due to closing of the right operculum fold, and left external gill shortened. The larva was about 7.24 ± 1.83 mm in length (Fig.15.24).

Stage 25 -Spiracle form on left side: After 3 days and 15 hrs, left operculum fold was also closed and completion of spiracle took place on the ventro-medial position. Mouth shifting to anterior tip of body and the tadpole became transparent and measured 9.25 ± 2.46 mm (Fig.15.25).

Stage 26 -Hind limb bud < ½ its diameter: The tadpole measured 14.31 ± 2.57 mm in length and hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 5 days 22 hrs. The length of the limb bud was wider than long. Melanophores were observed to be dispersed on the dorsal side of the body and tail region. No keratodonts were observed on the mouth region (Fig.15.26a-c).

Stage 27 -Length of limb bud ? ½ its diameter: The length of the limb bud equaled to half of its diameter was observed after 6 days and 18 hrs. The tadpole measured 18.26 ± 1.82 mm in length. Dispersion of melanophores was found to be comparatively denser than in the earlier stage (Fig.15.27).

Stage 28 -Length of limb bud ? its diameter: Length of limb bud equaled to its diameter on the 8th day and the total length was 20.49 ± 0.70 . In this stage melanophores were found to be denser in distribution, and the oral structure was found to be the same as in the earlier stages (Fig.15.28).

Stage 29 -Length of limb bud ? 1 ½ its diameter: On the 10th day, the limb bud becomes more elongated and it was about one and half of its width. The tadpole total length was 20.51 ± 1.03 mm (Fig.15.29).

Stage 30 -Length of limb bud = twice

its diameter: The limb bud further increased in length and becomes conical. The length was about twice its width, and the tadpole length was 20.54 ± 0.97 mm. Patch of pigments was observed on the limb, and there was no change in the oral structure. It was observed after 12 days (Fig. 15.30a-c).

Stage 31 -Foot paddle: A spatula-shaped limb bud was observed on the 14th day. Total length of the tadpole was 22.51 ± 0.76 mm (Fig. 15.31).

Stage 32 -First interdigital indentation: First indentation on the interdigits separated the 4th and 5th toes on the 16th day. At this stage, the tadpole length was about 23.64 ± 1.56 mm (Fig. 15.32).

Stage 33 -Second interdigital indentation: After indentation between toes 5-4, second interdigital indentation was observed between the 3rd and 4th toes. This stage was observed on the 18th day and the tadpole measured 24.66 ± 0.83 mm in length (Fig. 15.33).

Stage 34 -Third interdigital indentation: After 19 days, the margin of foot paddle became indented between toes 5-4, 4-3 and 3-2 separating the prominence of 2nd, 3rd, 4th and 5th toes. At this stage the thigh, shank and ankle with foot segment were well demarcated each other and the tadpole attained 25.32 ± 0.82 mm in length. Melanophore pigmentations became more prominent (Fig. 15.34a-c).

Stage 35 -Fourth interdigital indentation: Within 21 days where the total length of tadpole was about 26.49 ± 1.65 mm, indentation was seen between toes 1st and 2nd and all five toes were separated from each other. Melanophores were found to be more dispersed (Fig. 15.35).

Stage 36 - Separation of 5-4 and 4-3 toes: After 23 days, the total length of tadpole was about 27.68 ± 0.67 mm. The 3rd, 4th and 5th toes were separated, while the 1st and 2nd toes were still joined. Melanophores were seen to be more concentrated in the distal part (Fig. 15.36).

Stage 37 -Toes separation completed: All toes were completely separated and slightly elongated after 28 days. At this stage the average total length of tadpole

was about 28.27 ± 1.29 mm (Fig. 15.37).

Stage 38 -Appearance of Metatarsal tubercles: Metatarsal tubercles showed its appearance at the base of 1st toe after 31 days, and the total length of tadpole was about 28.56 ± 1.34 mm. Toes are free and no webs are seen in between digits (Fig. 15.38).

Stage 39 - Appearance of Subarticular patches: Subarticular tubercles first appeared as patches on the ventral digits after 33 days, and the total length of tadpole was about 29.42 ± 1.53 mm (Fig. 15.39).

Stage 40 - Completion of foot tubercles: After 35 days, toes were with fully developed subarticular patches with rudimentary webbed. The total length of tadpole was about 29.87 ± 1.26 mm (Fig. 15.40).

Stage 41 -Disappearance of Vent tube: After 38 days, the vent tube disappeared and the protruded fore limb could be seen on either side of the lateral body. The total length of tadpole was about 29.64 ± 1.72 mm (Fig. 15.41).

Stage 42 -Emergence of fore limbs: After 40 days, fore limbs emerged and the total length of tadpole was about 28.78 ± 0.88 mm (Fig. 15.42a-c).

Stage 43 -Mouth between nostril and eye: Resorption of tail started to regress after 43 days and the length of tadpole was about 23.28 ± 1.74 mm. The lateral margin of mouth reached between nostril and eye (Fig. 15.43).

Stage 44 -Mouth beneath eye: There was further widening of mouth after 45 days and the tail was greatly reduced and measured 15.53 ± 2.04 mm. Formation of the tongue took place (Fig. 15.44).

Stage 45 -Mouth posterior to eye: After 46 days, resorption of the tail was completed and only a stub remained, while the tadpole measured 12.16 ± 0.64 mm in length. And the mandible extended beyond the eye. Tongue had fully developed (Fig. 15.45).

Stage 46 -Metamorphosis completed: After 46 days, the tail was completely resorbed and the frog-let looks like an adult and measured 9.07 ± 0.47 mm (Fig. 15.46a&b).

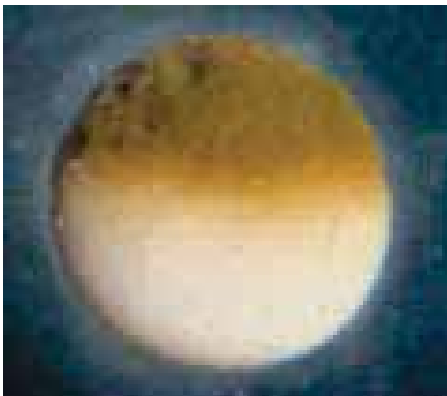


Figure 15.1 : Stage 1 Fertilization
Fig.15.2: Stage 2 Gray Crescent

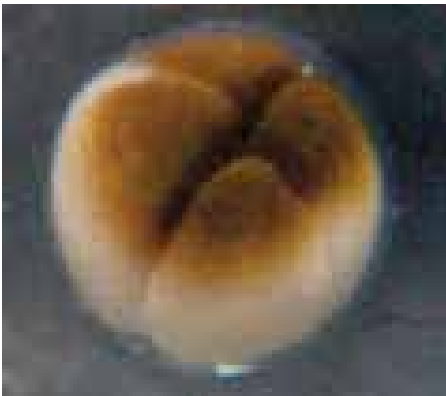
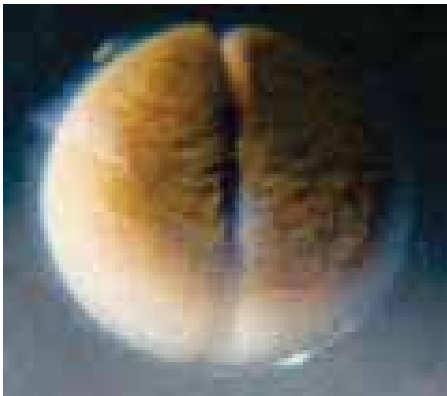


Figure 15.3 : Stage 3: 2-cell
Fig.15.4: Stage 4: 4-cell

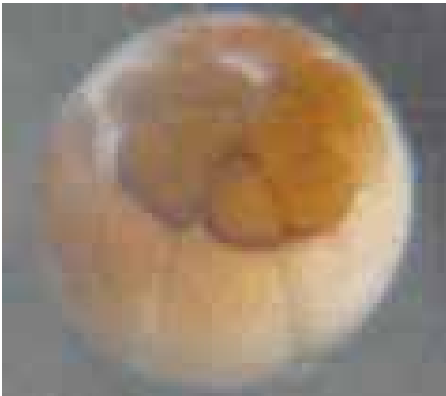


Figure 15.5 : Stage 5: 8-cell
Figure 15.6: Stage 6: 16-cell



Figure 15.7: Stage 7: 32-cell

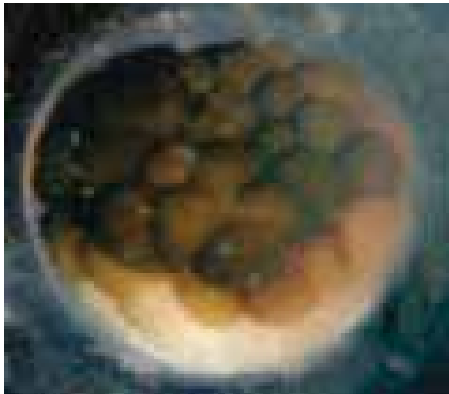


Fig.15.8: Stage 8: Mid Cleavage



Figure 15.9 : Stage 9: Late Cleavage



Figure 15.10: Stage 10 Dorsal Lip



Figure 15.11 : Stage 11: Yolk Plug



Figure 15.12: Stage 11: Yolk Plug



Figure 15.13: Stage 13: Neural Plate



Figure 15.14: Stage 14: Neural Folds



Figure 15.15 : Stage 15: Rotation
Fig.15.16: Stage 16: Neural Tube



Figure 15.17 : Stage 17: Tail Bud
Fig.15.18: Stage 18: Muscular Response



Figure 15.19 : Stage 19: Heart Beat
Fig.15.20: Stage 20: Gill Circulation



Figure 15.21 : Stage 21: Cornea Transparent
Fig.15.22: Stage 22: Fin Circulation

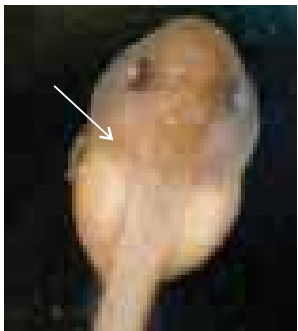
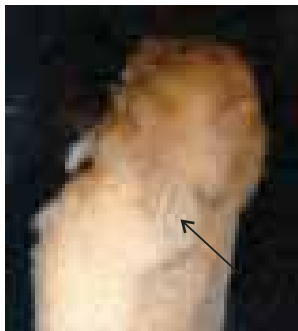


Figure 15.23 : Stage 23:
Operculum covers gill base

Fig.15.24: Stage 24: Left Gill

Fig.15.25: Stage 25: Spiracle
Forms on Mid-Ventral



Figure 15.26a : Stage 26: Dorsal
view

Fig.15.26 b: Stage 22: Fin
Circulation

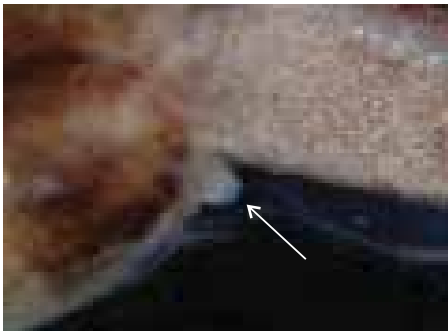


Figure 15.26 c : Stage 26: $L < \frac{1}{2}D$

Fig.15.27 : Stage 27: $L \geq \frac{1}{2}D$



Figure 15.28 : Stage 28L $\geq D$

Fig.15.29 : Stage 29L $\geq 1\frac{1}{2}D$

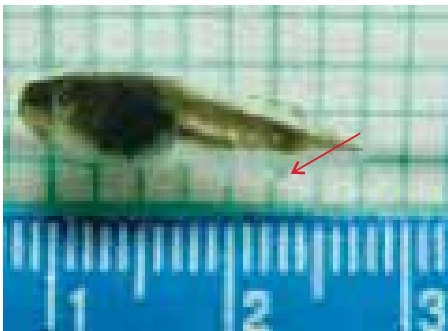
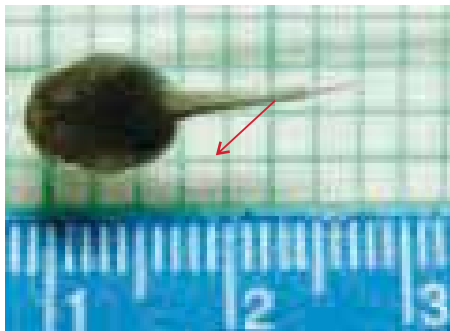


Figure 15.30 a : Stage 30: Dorsal
view

Fig.15.30 b : Stage 30: Lateral
view



Figure 15.30 c: Stage 30:
 $L \geq 2D$
Fig.15.31: Stage 31: Foot Paddle

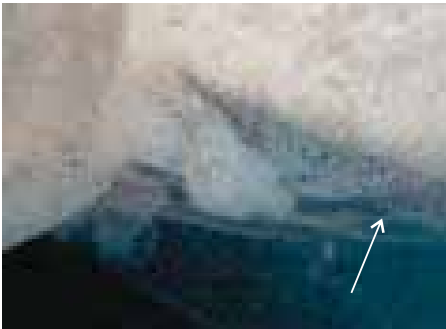
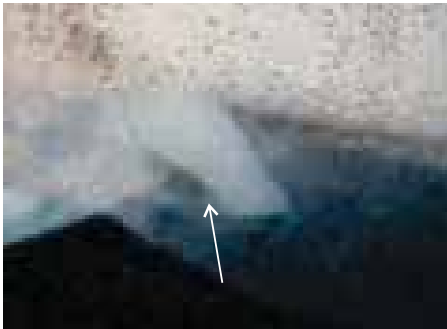


Figure 15.32 : Stage 32:
Indentation 4-5
Fig.15.33: Stage 33:
Indentation 3-4

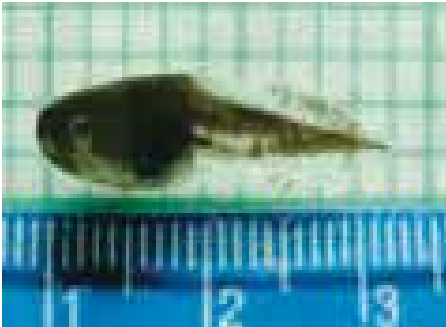
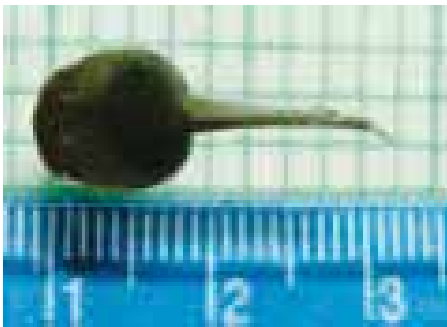


Figure 15.34 a : Stage 34: Dorsal
view
Fig.15.34 b: Stage 34: Lateral
view

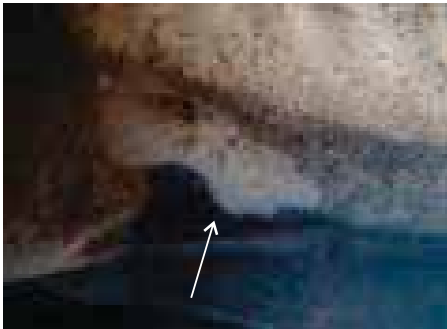


Figure 15.34 c: Stage34:
Indentation 2-3
Fig.15.35: Stage 35:
Indentation 1-2



Figure 15.36 : Stage 36: Toes 3-5
Separated
Fig.15.37: Stage 37: All Toes
Separated

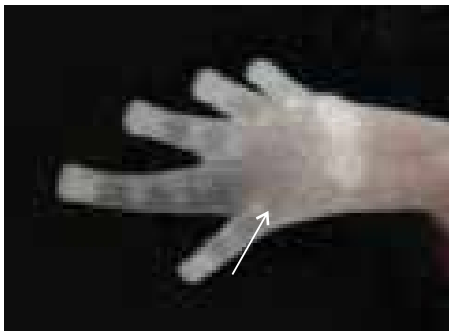


Figure 15.38 : Stage 38:
Metatarsal tubercles
Fig.15.39: Stage 39:Sub-articular
patches

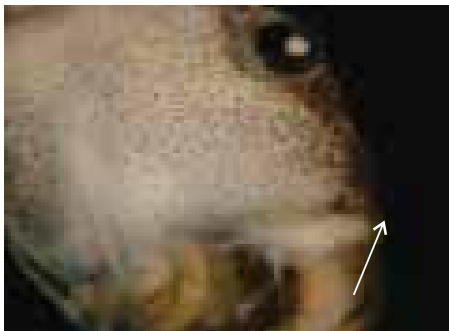
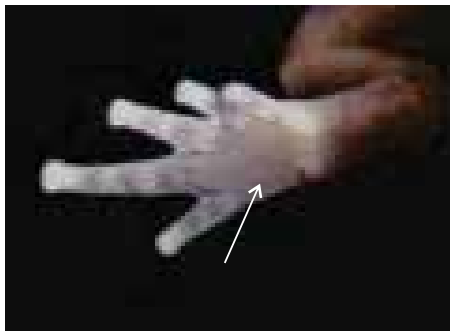


Figure 15.40 : Stage 40:
Foot Tubercles
Fig.15.41: Stage 41:Fore Limbs
Visible



Figure 15.42 a & b : Stage 42: (a.)
Right Fore Limb Emerge first (b.)
Fore Limbs Emerge

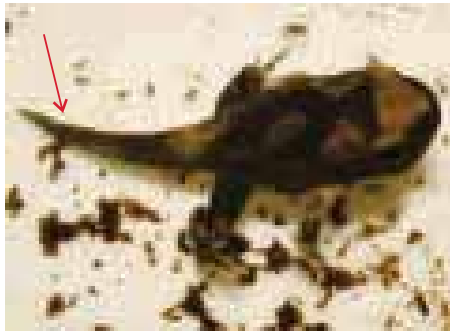


Figure 15.43 : Tail Atrophies
Fig.15.35: Stage 44: Tail Greatly
Reduced



Figure 15.45 : Stage 45: Tail Stub
Fig.15.46a: Stage 46:
Metamorphosis Complete



The development and metamorphosis was therefore completed within about $47 (\pm 2)$ days in *Kaloula pulchra* at temperature between 16°C and 28°C in the natural environment. In the laboratory also, the pattern and duration of development and metamorphosis was observed to be more or less the same during the breeding season.

Food and feeding in relation to oral structures and intestines:

Qualitative analysis of gut contents of the tadpole of *Kaloula pulchra* revealed that the larvae started feeding from stage 25 onwards. The food items found at different stages of development of the tadpoles of *Kaloula pulchra* are shown in Table 5.

Larval foods of *Kaloula pulchra*:

Stage 25: Analysis of the gut contents of stage 25 revealed phytoplankton namely, Diatoma, Navicula, Stauroneis and Tabellaria (Bacillariophyceae), Cosmarium, Crucigenia and Scenedesmus (Chlorophyceae), Anabaena, Nostoc and Oscillatoria (Cyanophyceae), Arcella and Cryptomonas (Cryptophyceae).

Stages 26 – 30: Though the gut contents of stage 25 and stages of hind limb bud development are very similar, apart from the previous stage, phytoplankton like

Pinnularia (Bacillariophyceae), Closterium (Chlorophyceae), and Euglena (Euglenophyceae) and a zooplankton Lecane were also observed.

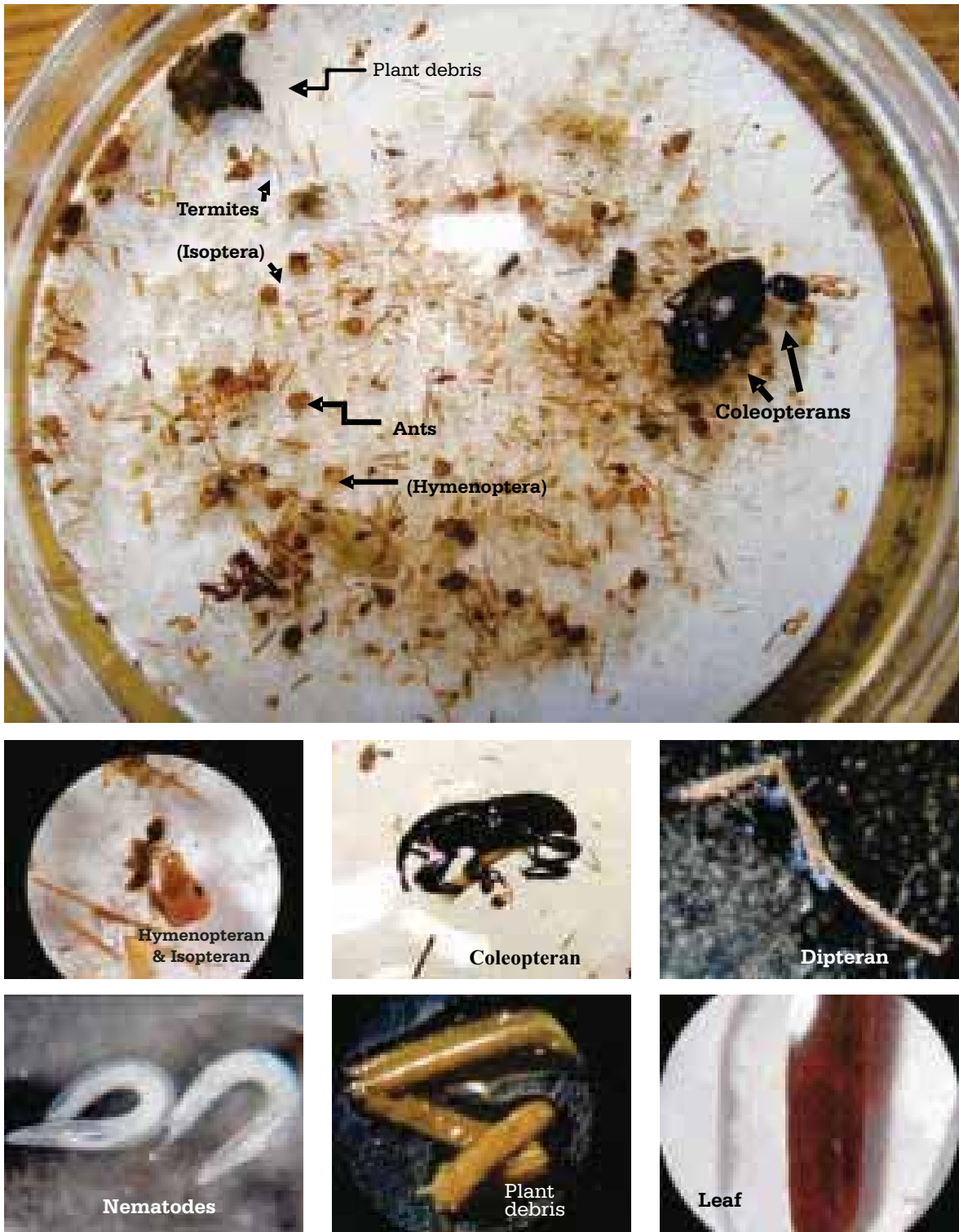
Stages 31 – 41: During toe development, in addition to the food items of earlier stages, gut content analysis revealed Phacus (Euglenophyceae) and a zooplankton Paramecium.

Stages 42 – 46: After the emergence of fore limb, the oral apparatus of tadpoles start to degenerate which consequence with ceased feeding. From stage 46 onwards, the animal starts to feed on other small invertebrates.

Adult foods of *Kaloula pulchra*:

The stomach content of the adults ($N=30$) was analyzed and it was found that the adults feed mostly on small insects in which Isopterans (wingless and winged termites) shows the highest, followed by other insects Coleoptera (e.g. beetle, Curculionidae), Dipteran flies, Hymenoptera (e.g. winged and wingless ants), Orthopteran nymphs, and also include nematodes and Annelids (e.g. earthworm). Pieces of leaves and twigs are also recovered (Fig. 16).

Figure 15.46 b : Emerging froglets and metamorphs in the field.



Oral structure of the tadpoles of *Kaloula pulchra*

From the present study based on the light and electron micrographs, the larvae of *Kaloula pulchra* show no keratinized structures, such as jaws and labial teeth; in addition they have a terminally-oriented, as opposed to an antero-ventrally oriented mouth (umbelliform) known as semicircular labial flap (Figs.17a&b and Fig.18). They are filtering food particles throughout the water column. At stage 42, the tadpole stopped feeding and the umbelliform mouth part is degenerated and gradually transformed in to adult mouth (Fig. 19).

Fig. 16: Food items found in the gut of adult Kaloula pulchra

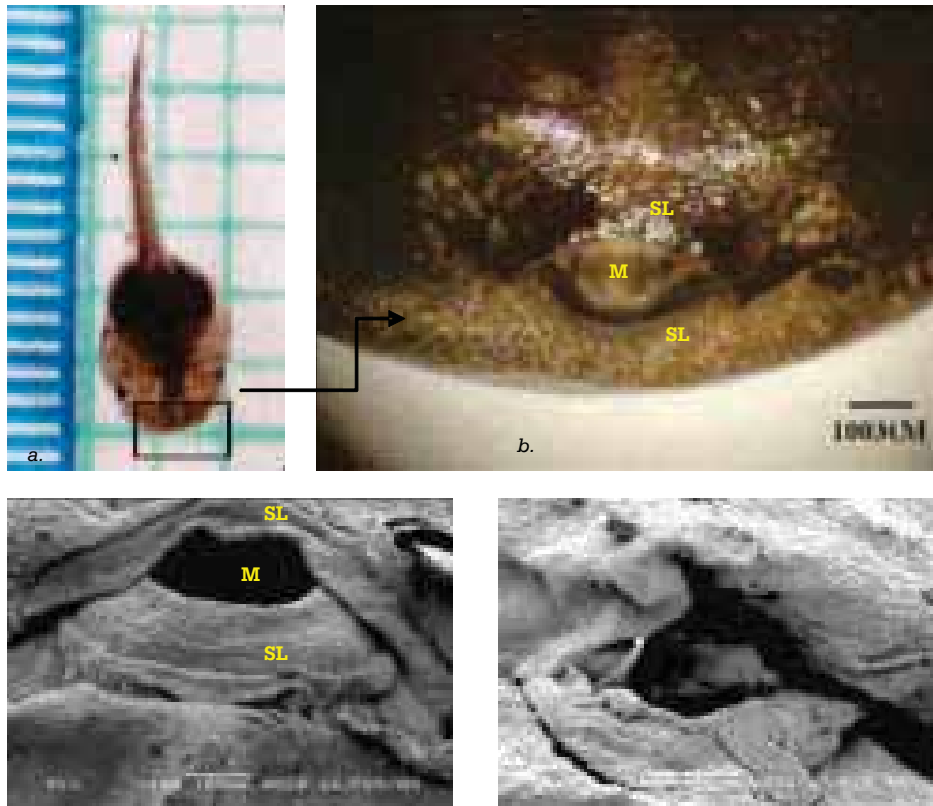


Figure 17 a & b: Light micrographs of stage-25 of *Kaloula pulchra* (a.) dorsal view of whole body, and (b.) oral apparatus. SL: Semicircular labial flap; M: Mouth.

Figure 18 : Scanning electron micrograph of oral apparatus at stage 25 of *Kaloula pulchra*.

Figure 19 : Scanning electron micrograph of degenerating oral apparatus at stage 42 of *Kaloula pulchra*. Histological study on the intestines of tadpole and adult:

The present investigation showed that the tadpole intestine consists of two spiral coils: the outer coil (duodenum and anterior ileum) reverses direction at the switchback point followed by the inner coil (posterior ileum and colon), which terminates at the rectum. The findings indicated that the intestines of tadpole remodeled during metamorphosis. The morphological changes that take place during intestinal remodeling are more drastic and the intestinal epithelium is a complex structure that provides an enormous luminal surface area for efficient food processing and absorption, the primary function of the organ. The tadpole intestine has a long and simple structure. It consists of layers of columnar epithelium surrounded by thin layers of muscles with little intervening connective tissue.

The length of tadpole intestine was increased from stage 25 to stage 40, there is significant positive correlation between the tadpole length and the gut length at the 0.01 level (2-tailed), where $p < 0.01$ (Table 7). Histological study revealed that the primary epithelia are surrounded by submucosa which is further enclosed by a thin serosa.

The intestine attained its maximum length (62.75 mm) at stage 40 where the tadpole reached a maximum total length, 29.92 mm (Table 6). From stage 40 to stage 46, Table 8 showed that the lengths of total body and intestines greatly reduced, showing positive correlation ($p = 0.017$) at the 0.01 level (2-tailed). Within 9 - 12 days the *Kaloula pulchra* tadpole intestine (from stage 40 - stage 46) reduced its length by about 80%. At stage 46, the primary epithelium (PE) degenerates and the secondary epithelium (SE) are formed. Intestinal folds (IF) appear as several circular folds lined by mucus membrane.

Discussion

Kaloula pulchra in Mizoram

The field survey and collections revealed that *Kaloula pulchra* preferred ephemeral pools and backwaters of stream or river with some vegetation surrounding it as their habitat. These temporary pools and water with leaf litter and vegetation provides excellent habitat for breeding and development of these microhylid frogs. *K. pulchra* was mostly found to inhabit

disturbed areas near human habitation as observed by Inger (1999). *K. pulchra* was first reported from India by Romer (1949) from Nagaland state, North Eastern India. It was subsequently reported from Tinsukia and Cachar District, Assam state, North Eastern India (Dutta 1997; Dey et al. 2000) and also Cherrapunjee, East Khasi Hills District, Meghalaya, (Hooroo et. al. 2002). Pawar and Birand (2001) reported from three localities, Dampa, Ngengpui and Palak dil of Mizoram. Sailo et al. (2005) reported from only one locality of Mizoram (Sihhmui). Sarkar et al. (2002) reported from Tripura. The distribution of *K. pulchra*, reported in various literatures (Sekar 1991; Naik et al. 1993), from southern India to Bengal and also to western India probably refer to another species of the genus, *K. taprobanica* (Sengupta et al. 2009). However, the present investigation provided more information about the distribution of the species collected from 10 different areas in Mizoram. Outside India, *K. pulchra* is reported from Indochina, Bangladesh, Indonesia (Sumatra, Borneo, and Sulawesi), Myanmar and Thailand to southern China (southern Yunnan, Guangxi, Hainan and Guangdong, and Taiwan), south to

Singapore and introduced into Philippines (Luzon, Cebu, Marinduque, Mindanao, and Palawan) (Orlov et al. 2002; Frost 2017). It thus, seems that *K. pulchra* inhabits various sites of Mizoram irrespective of the altitudes ranging from 180 m - 963 m asl, indicating that *K. pulchra* has diverse adaptations to live in low and mid altitudes. In Vietnam, Orlov et al. (2002) also reported from seaside lowlands and montane regions, ascending up to 1200 m asl.

Inger (1999) included *K. pulchra* among a few species that seem to be most common in severely disturbed environments in southern Asia, where they form large populations and rarely seen in forests. Zug et al. (1998) observed *K. pulchra* predominantly in the forest, and all breeding males in forest or forest-edge pools, although it is a known resident of garden and landscaped sites in villages and towns. Das (2008) reported that although *K. pulchra* is a burrowing frog, the individuals of the species climbs well and often seen 30 cm - 1 m above surface and individuals were recorded from tree hole at 1 - 2 m above. Sengupta et al. (2009) also reported one individual at 3 m height while climbing a tree during a heavy shower.

Waterpools created on rocky stream bed are often used as breeding micro habitats by the studies species



Morphometric measurements and Sexual dimorphism

Snout-vent length (SVL) of *Kaloula pulchra* in this study (male: SVL = 61.92 ± 0.49 mm; N = 50 and female: SVL = 64.60 ± 0.51 mm; N = 50) are more or less similar to those of Western China with SVL = 69 mm (Liu, 1950), Thailand specimens: male SVL = 65 mm and female SVL = 70 mm (Taylor 1962); 61 mm - 70 mm from Myanmar (Zug et al. 1998); 80 mm - 85 mm (Chanda 2002), male: 55 mm and female: 58 mm from Meghalaya (Hooroo et al. 2002); male: 56 mm - 64 mm and female: 69 mm from Sihhmui, Mizoram (Sailo et al. 2005); 85 mm by Ahmed et al. (2009) and 73 mm (Sengupta et al. 2009).

Ecological factors at breeding sites

From the present data, ANOVA test on the breeding season against environmental factors (air temperature, relative humidity, rainfall, water temperature and pH) did not differ significantly, where $p > 0.05$. It was suggested that only the onset of monsoon stimulates the animals to emerge from their subterranean retreats and strong choruses of breeding aggregations have been heard following prolonged rains which lasted several days. Heyer (1973), Aichinger (1987), Gascon (1991b), and Donnelly and Guyer (1994) suggested that the single physical factor of rainfall distribution regulates anuran reproductive patterns in tropical areas characterized by a pronounced dry season. In Singapore, Berry (1964) also observed that the aggregation of *Kaloula pulchra* at their breeding grounds depended entirely on rain. In Assam, Sengupta et al. (2009) reported that during April-June, calling aggregations of *K. pulchra* were recorded from swampy or temporary waterlogged areas in and around human habitations as well as from forest edges. The breeding period of *K. pulchra* in study area coincides with population from tropical Thailand (Heyer 1973), and almost similar with other *Kaloula* species tropical microhylids such as *K. assamensis* between June and July (Ahmed et al. 2009), *K. baleata* at the beginning of the rainy season (Diesmos et al. 2004), *K. borealis* around June and July (Fei 1999), *K. conjuncta* stimulated by rains in all months except

January and March (Alcala 1962), *K. indochinensis* during heavy rains (Chan et al. 2013), but quite different from the pattern of continuous rainfall-dependent breeding in *K. pulchra* and other microhylids reported from temperate region where climatic condition is not seasonal as reported by Berry (1964). However, males calling activities after June agreed with the observation made by Sengupta et al. (2009). The present observation on the breeding sites in small pools, usually seasonal rain pools, or ponds was supported by other reports (Leong and Chou 1999; Kuangyang et al. 2004; Sengupta et al. 2009). Observation on the males calling in the water at the edge of pools was also reported by Zug et al. (1998), and the axillary amplexus was like those of other microhylids (Leong and Chou 1999; Heying 2003).

In amphibians, reproductive activities are greatly affected by the changing climatic factors such as the temperature, rainfall, day light length and relative humidity. This factor may indicate water availability, which generally stimulates anuran reproduction (Telford and Dyson 1990; Gascon 1991a). Gopalakrishnan and Rajasekarasetty (1978) reported that studies on the annual reproductive behavior of the temperate, subtropical and tropical species of amphibia indicate that the internal hypophysio-gonadal rhythm is under the mark influence of a variety of external factors. A complex pattern of the external stimuli is required to induce the breeding behavior, no single stimulus is adequate and yet temperature and rainfall are important factors to reckon (Amoroso and Marshall 1960). Explanations of temporal variation in breeding activity in anurans often implicate rainfall because of the predominant external mode of fertilization, and the aquatic larval phase generally makes the availability of free water an essential requirement for breeding (Duellman and Trueb 1994).

Breeding behaviour and mating calls

The advertisement call of *K. pulchra* emitted in a series consisted of a single note as also observed by Heyer (1971). The notes lasted 450.8-620.3 ms and were composed of a



series of 36-45 pulses. The frequency spectra had a dominant band at 1265 Hz. The call duration of *K. pulchra* from Thailand (Heyer 1971) ranged from 560 to 600 ms, consisted of 18-21 pulses/call, and the frequency spectra had a dominant band at 250 Hz. This analysis shows a little difference with others except in the call duration.

Several studies have shown that social as well as environmental factors influence some call characteristics, such as: dominant frequency, number of pulses, duration, and repetition rate of the note (Wells 1988; Wilczynski and Ryan 1999). The differences in the call parameters may reflect geographic variation, as described in *Rana ridibunda* (Schneider 1973; Nevo and Schneider 1983; Kuhn and Schneider 1984; Schneider and Sofianidou 1985), *Bombina orientalis* (Schneider et al. 1986), and *Microhyla ornata* (Hiremath 1991). In several species, body temperature has a greater effect on call length and rate than body size or condition (Rome et al. 1992; Howard and Young 1998; Castellano and Giacoma 1998;

Tarano 2001). In anurans, spectral call properties, such as dominant or fundamental frequency, are usually negatively correlated with body size because of morphological constraints on the sound producing apparatus (Martin 1972). A number of factors have been invoked to explain geographic variation in frog calls including reinforcement (Butlin 1987; Loftus-Hills and Littlejohn 1992; Howard and Gregory 1993), changes in the acoustic environment (Ryan 1988), or a divergence associated with morphological change over the geographic range of the species (Nevo 1973). Current evidence suggests that advertisement calls vary between and within populations of the same species (e.g. Ryan et al. 1996; Gergus et al. 1997; Smith et al. 2003). Other studies have found considerable intraspecific variation in the advertisement call of frog species that inhabit broad geographic areas with a range of environmental and climatic conditions (Nevo and Capranica 1985; Ryan and Wilczynski 1991; Ryan et al. 1996; Hasegawa et al. 1999). Geographic

Kaloula sp., an intermediate between *U. taprobanica* and *K. assamensis*.
Photo Credit: Abhijit Das

divergence in advertisement call structure can be associated with genetic subdivision (Ryan and Wilczynski 1991; Ryan et al. 1996; Castellano et al. 1998).

Oviposition sites

During the study period, every year *K. pulchra* used the same microhabitat of the study sites for breeding and oviposition unless it was destroyed by natural calamities (floods, minor landslides, etc.) and anthropogenic activities. This shows that *K. pulchra* is very selective in microhabitat choice and the oviposition sites were mainly temporary rock-pools or water holes which accord with the observation of Heyer (1973). This might be in order to avoid tadpoles predation, naturally the smaller rock-pools totally lack predators of tadpoles whereas others support high densities of various predator species as in the case of common frogs in Southern Finland (Laurila and Aho 1997). The larvae of pool anurans are unable to leave the sites selected by their parents and therefore there should be particularly strong selective pressure on adult females for the ability to discern oviposition sites in which offspring survival is expected to be high (Iwai et al. 2007). According to Heyer et al. (1975) and Skelly (1997), abiotic factors (e.g. water body duration) are more important than biotic factors (e.g. competition, predation) for species' reproductive success in temporary water bodies, but predation is not independent of hydroperiod.

Clutch Size

The clutch sizes of *K. pulchra* from Mizoram (range 363 - 576), much less than findings of Berry (1964) who counted 1574 - 6330 per a single specimen. Leong and Chou (1999) reported 2 - 3 dozens or even up to hundreds from the same country, Singapore. Mohanty-Hejmadi et al. (1983) reported the number of eggs correlates with the female's nutritional state. Moreover, Ritke et al. (1990) and, Morrison and Hero (2003) reported that clutch size and breeding phenology may vary over the geographic range of a wide-ranging species which may lead to variation in population dynamics.

Clutch size Vs Female body size

No correlation was, however, observed between female body size and clutch size ($r=0.187$; $p=0.167$). Although some workers reported that the clutch size and female size are correlated in some species, *Rana temporaria* (Cummins 1986; Joly 1991), *R. dalmatina* (Ponsero and Joly 1998), *Microhyla ornata* (Matsui and Ota 1984). Other workers reported no correlation between the same e.g. *Rana* (*Hoplobatrachus*) *tigerina* (Dutta and Mohanty-Hejmadi 1976); *Heleioporus albopunctatus* (Davies and Roberts 2005). Dziminski (2000) reported that only three of 11 species of Australian frog species showed a positive relationship between clutch and body size.

Development and Metamorphosis

By conducting studies on the embryonic development and metamorphosis of *K. pulchra* both in the natural and laboratory conditions with the water temperature ranged between 14.5°C - 29°C and 16°C - 28°C respectively, it was found that the completion of life cycle occurred within 47 ± 2 days. Hatching of the larva was observed in the stage-20 after 29:30 hrs when the larva measured about 4.33 mm in length. The dependent of life cycle in the temporary rock-pools and ephemeral water bodies is in agreement with Heyer (1973), and Leong and Chou (1999). The hatching period and duration of life cycle was very close to other Indian microhylids like, *Uperodon systoma* that hatched in about 30 hr after fertilization and completed the life history within 51 days in the laboratory condition (Mohanty-Hejmadi et al. 1979), *Microhyla ornata* that also hatched in 34 hrs and accomplished its life cycle within 49 days (Mohanty-Hejmadi et al. 1980) and *Uperodon variegata* hatched in 28 - 30 hrs with 32 days life cycle duration (Dutta et al. 1990-91). Other *Kaloula* species like, *K. conjuncta* hatched at 39 hours and completed life cycle within 82 to 87 days (Acala 1962). The timing of metamorphosis in this study (47 ± 2 days) is more than findings of Leong and Chou (1999) (4 weeks) in the temperate region of Singapore and the average diameter of eggs (1.48 mm) in our observation was slightly smaller than

their reports (1.5 mm - 2 mm). In *K. borealis*, tadpoles matured within 30 days (Fei 1999). Most of the larval characteristics are in agreement with their observation except for the size of larger tadpoles in the present study which can be explained by the fact that low temperatures retard differentiation more than growth, thereby increasing stage-specific size (Smith-Gill and Berven 1979). As a result, larval anurans grown at cold temperatures have prolonged developmental periods but they are also larger as metamorphs than conspecifics grown at warmer temperatures. This phenomenon makes up one of the most general rules for ectotherms (Atkinson 1994 and 1996).

The breeding and life cycle of *K. pulchra* is comparatively shorter and it might be referred to as 'explosive breeder' used by Ritcher and Seigel (2002) for those species that breed in temporary water bodies where metamorphosis and development is completed within a short hydroperiod. The observed rapid developmental rates might be advantageous for these microhylid species in ephemeral habitats, which allow larvae to metamorphose quickly and escape desiccation and reduce exposure to aquatic predators and diseases as reported also in other frog species (Low 1976; Newman 1992; Denver 1997, 1998). Tadpoles from small temporary ponds have been reported to spend more time feeding and to develop faster than tadpoles from large permanent ponds, where the larvae spend more time hiding from predators, and, as a consequence develop more slowly (Peltzer and Lagmanovich 2004).

In the present study, the pH of water from the time of egg laying till the completion of metamorphosis at the study sites I and II ranges from 6.0 - 6.7 and 6.1 - 6.7, respectively. It is suggested that this pH range (6.0 - 6.7) seems to be the optimal pH of water for breeding and development of *K. pulchra*. Freda and Dunson (1985) that, low pH can have important physiological as well as ecological consequences for amphibian populations. Pough and Wilson (1977) also reported that amphibian embryos are more sensitive to low pH at higher temperatures. Low environmental pH can also cause death of amphibian embryos by a selective

effect that leads to constriction of the extra-embryonic membranes and severe curling of the embryo (Dunson and Connell 1982). Although some workers have suggested that some populations of certain species of frogs show a greater tolerance to low pH (Gosner and Black 1957; Clark and Lazerte 1987), this is not a general phenomenon (Clark, 1986). Very little is known about the potential of amphibians to adapt to low pH conditions (Andren et al. 1989). Several experimental studies revealed negative effects of acid water on embryos and larvae of different amphibian species (Andren et al. 1988; Rowe et al. 1992) and also reduced the survival probability as well as increased the frequency of developmental anomalies in some amphibian species (Pahkala et al. 2001). During development, the embryonic stage appears to be most sensitive: low pH leads to a denaturation of the hatching enzyme (Urch and Hedrick 1981) and subsequently to deformations of the embryo and high embryonic mortality.

Larval foods

Larvae of *K. pulchra* are nektonic filter-feeders. Observation on the present results showed that tadpoles started feeding from stage 25 onwards. During the early stages of feeding, they feed mostly on detritus and plant materials and during the later stages of feeding they consumed both phytoplankton and zooplankton. Tadpoles soon stop feeding at stage 42 and after metamorphosis the froglet start feeding on a carnivorous diet. As they were mainly collected from small rock-pools within a short period, Bacillariophyceae and Cyanophyceae (especially *Anabaena*) are comparatively more abundant than other food items. The preference of Bacillariophyceae and Cyanophyceae in the food habits of this species may be that the only available food items in their microhabitat that enhanced the faster developmental rate within a short duration. From the observation of Pryor (2003), it was evident that diatoms alone are sufficient for the growth and development of tadpoles and diatoms contain more calories (in the form of fat and protein) than other algae and hence they are a preferred food (Kupferberg 1997). Moreover, it was observed that the

filamentous blue-green alga *Anabaena* promoted better growth in Bullfrog tadpoles (*Rana catesbeiana*) than the other algal species (Pryor 2003).

In *K. pulchra*, the range of food particle size, as well as the qualitative composition of the diet in tadpoles, is highly variable, and is linked to food availability. Therefore, the present investigation showed availability and composition of food directly affects performance of anuran larvae as reported by Beck (1997), Brown and Rosati (1997), and Kupferberg (1997). However, Heyer (1973) and Inger (1986) mentioned interspecific variation in the sizes of food particles ingested.

Oral apparatus

Variation on oral morphology of different anuran species agrees with the study that the structure of the mouth and buccal cavities of anuran larvae are highly adaptive and correlated with feeding ecology (Wassersug 1976). Khan and Mufti (1994) reported that oral morphology of anuran tadpoles differ specifically reflecting adaptive radiations of each species to exploit different parts of the available food base in the pond ecosystem. The present findings reported that the LTRF in *K. pulchra* is consistent up to metamorphic

stages. Absence of jaw sheath and keratodonts in the tadpoles of *K. pulchra* agrees with a report made by Inthara et al. (2005), where *Microhyla berdmorei*, *M. ornata*, *M. butleri*, *M. pulchra*, *Micryletta inornata*, and *Glyphoglossus molossus* are also included. Rao (1933) also reported the total absence of the keratodonts in the tadpoles of *Kaloula* and *Microhyla*. Das and Coe (1994) also reported the absent of denticles in *Microhyla rubra*, *M. ornata* and *Uperodon systoma*. In the present observation, the umbelliform disc tadpole of *M. berdmorei* has a large, in folded semicircular structure at each corner of the mouth that appears derived from the lower labium as it is also seen in the tadpoles of *Microhyla heymonsi* (Altig and McDiarmid 1999) and *M. ornata* (Khan and Mufti 1994). Altig and Mc Diarmid (1999) reported that an umbelliform or upturned oral disc appears as convergent trait in tadpoles of some arthroleptids, dendrobatids, hylids, mantelline rhacophorids, megophryids, and microhylid microhylids. Most umbelliform tadpoles occur in the backwaters of lotic systems. Tooth rows and jaw sheaths are reduced to absent in these forms, and large ridge-like papillae that project radially from the mouth are common.

Tamdil-A typical amphibian breeding habitat in Mizoram.



Adult Foods

The stomach content suggested that, insects belonging to Isoptera, especially to family Termitidae (non winged forms) and winged termites were the most important food of these frogs. The abundance of termites in their food items presumably due to emergence coincides with the availability of termites following rains. Emerson (1976) reported that burrowing frog populations usually inhabit concentrated food areas. The concentration on termites and lack of mud supports the view that *K. pulchra* mainly feeds at the surface rather than underground as observed in *Uperodon systoma* (Mohanty-Hejmadi and Acharya 1979).

The findings on the food composition consisted of terrestrial and aquatic insects. Terrestrial and aquatic insects have been reported as preferential anuran prey items in several studies conducted over the past 20 years (Toft 1980, 1981; Van Sluys and Rocha 1998; Anderson et al. 1999; Cogalniceanu et al. 2001). The occurrence of leaves and other debris in the stomach might have been ingested incidentally with the prey as also observed by Mathew and Andrews (2001). The present results on the comparatively smaller size foods among adult microhylids might be reflected by their head shape, which is important in the number and size of prey they consumed. A shorter jaw may facilitate a faster feeding cycle and may be advantageous for an animal that needs to consume large quantities of relatively low quality prey such as ants. Present observation on the foods of *K. pulchra* shows that, although microhylids are ant specialist, ants and termites are not the only prey they consume, but also small beetles, dipterans, small orthopterans and so on as reported by Parmelee (1999). Several other studies have also demonstrated ontogenic changes in diet (Christian 1982; Donnelly 1991; Flowers and Graves 1995; Labanick 1976; Lima 1998).

Histological study on the intestines of tadpole and adult

Shortening of the intestine during spontaneous metamorphosis is observed by 80% in *K. pulchra* which is supported by the

shortening of premetamorphic gut length to its one third by completion of metamorphosis and the shortening occurs uniformly along the intestine's length (Pretty et al. 1995). The abrupt shortening of the anuran gut during metamorphosis has been well documented. Schreiber (2005) reported that in one week, at the climax of metamorphosis, the intestine shortens 58-90% depending on the anuran species, 42.3% for *Phrynohyas resinifictrix* (Wilczyńska et al. 2004), 58.15% in *Rana temporaria*, 75% in the *Xenopus laevis* (Schreiber 2005), 82.2% in *Rana catesbeiana* (Janes 1934), 84% in *Rana catesbeiana* (Carver and Frieden 1977) and to as high as 90% in *Alytes obstetricians* (Dauca and Hourdry 1985). In the present study, shortening of the intestine during spontaneous metamorphosis accompanied by a change in a single layer of cuboidal epithelial cells into a complicated layers consisting of secondary epithelium, intestinal fold lined with numerous microvilli agreed with other anurans studied so far (Schreiber 2005). These changes in the cellular and tissue structures as well as shortening the length of the intestine are important for the transformation of aquatic omnivorous tadpoles in to terrestrial carnivorous frog.

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Table 1: *Kaloula pulchra* collection sites during the present survey

Sl. No.	Districts	Areas	Latitudes	Longitudes	Elevation(m asl)
1.	Aizawl	Dihmunzawl		23°43' N	92°40'E525
		LAD Picnic spot, Aizawal	23°42'58" N	92°43'52"E	783
		Mizoram University Campus	23°44' N	92°40'E	865
		Sihhmui	23°47'- 48'N	92°38'-39'E	180 - 184
		Tanhrlil	23°44'17" N	92°40'33"E	963
2.	Kolasib	Tuitun	23°58'N	92°41'E	308-326
		Kawnpui	23°56'N	92°41'E	910
3.	Mamit	Damparengpui, Dampa Tiger Reserve	23°43'06"N	92°24'47"E	418
		Lengpui	23°49'-50'N	92°37'E	390-400
		Vaipuanpho	23°42'N	92°38'E	339

Table 1: ANOVA table to test the significance of differences between air temperature, relative humidity, rainfall, water temperature, water and pH at the study site-I during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	2.920139	1	2.920139	0.274	0.604
	Within Groups	362.4323	34	10.65977		
	Total	365.3524	35			
Relative humidity	Between Groups	5.166667	22	0.234848	1.078	0.458
	Within Groups	2.833333	13	0.217949		
	Total	8	35			
Rainfall	Between Groups	26182.35	1	26182.35	0.807	0.375
	Within Groups	1102540	34	32427.65		
	Total	1128722	35			
Water temperature	Between Groups	5.133333	19	0.270175	2.316	0.087
	Within Groups	1.166667	10	0.116667		
	Total	6.3	29			
Water pH	Between Groups	2.788095	9	0.309788	1.764	0.139
	Within Groups	3.511905	20	0.175595		
	Total	6.3	29			

Table 2: ANOVA table to test the significance of differences between air temperature, relative humidity, rainfall, water temperature, water and pH at the study site-II during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Air Temperature	Between Groups	4.833333	22	0.219697	0.902	0.598
	Within Groups	3.166667	13	0.24359		
	Total	8	35			
Relative humidity	Between Groups	6.2	22	0.281818	2.035	0.094
	Within Groups	1.8	13	0.138462		
	Total	8	35			
Rainfall	Between Groups	8793.59	1	8793.59	0.275	0.603
	Within Groups	1087655	34	31989.85		
	Total	1096449	35			
Water temperature	Between Groups	0.114683	1	0.114683	0.048	0.828
	Within Groups	67.08532	28	2.395904		
	Total	67.2	29			
Water pH	Between Groups	3.333333	11	0.30303	1.839	0.121
	Within Groups	2.966667	18	0.164815		
	Total	6.3			29	

Table 3: Age and size of developing *Kaloula pulchra* embryos reared at 16°C – 28°C. (N= Total number of samples examined).

Sl.No.	Stage	Age	Size in mm (N= 10)
1.	Fertilization	0 hr	1.48 ±0.43
2.	Gray Crescent	0:25 hr	1.48 ±0.71
3.	2-cell	0:40 hr	1.51 ±0.64
4.	4-cell	1:05 hrs	1.52 ±0.38
5.	8-cell	1:55 hrs	1.50 ±0.27
6.	16-cell	2:50 hrs	1.51 ±0.53
7.	32-cell	5:00 hrs	1.52 ±0.84
8.	Mid Cleavage	6:55 hrs	1.53 ±0.52
9.	Late Cleavage	8:20 hrs	1.55 ±0.27
10.	Dorsal Lip	9:30 hrs	1.55 ±0.63
11.	Yolk Plug	11:15 hrs	1.59 ±1.45
12.	Late Gastrula	12:45 hrs	1.67 ±1.29
13.	Neural Plate	14:55 hrs	1.84 ±0.75
14.	Neural Fold	16:40 hrs	2.39 ±0.63
15.	Rotation	18:05 hrs	2.40 ±1.19
16.	Neural Tube	18:45 hrs	2.47 ±0.42
17.	Tail Bud	19:15 hrs	3.01 ±0.74
18.	Muscular Response	20:10 hrs	3.57 ±0.92
19.	Heart Beat	22:15 hrs	3.90 ±1.23
20.	Tail Elongation (Hatching stage)	1 day 5:30 hrs	4.33 ±1.62
21.	Cornea Transparent	1 day 11 hrs	5.63 ±1.35
22.	Tail Fin Circulation	1 day 21 hrs	5.93 ±1.02
23.	Operculum present	2 days 12 hrs	6.37 ±1.28
24.	Left Gill	2 days 23 hrs	7.24 ±1.83
25.	Spiracles Forms	3 days 15 hrs	9.25 ±2.46
26.	L < ½D	5 days 22 hrs	14.31 ±2.57
27.	L < ½D	6 days 18 hrs	18.26 ±1.82
28.	L < D	8 days	20.49 ±0.70
29.	L < 1½D	10 days	20.51 ±1.03
30.	L < 2D	12 days	20.54 ±0.97
31.	Foot Paddle	14 days	22.51 ±0.76
32.	Indentation 4-5	16 days	23.64 ±1.56
33.	Indentation 3-4	18 days	24.66 ±0.83
34.	Indentation 2-3	19 days	25.32 ±0.82
35.	Indentation 1-2	21 days	26.49 ±1.65
36.	Toes 3-5 Separated	23 days	27.68 ±0.67
37.	All Toes Separated	28 days	28.27 ±1.29
38.	Metatarsal tubercles	31 days	28.56 ±1.34
39.	Sub-articular patches	33 days	29.42 ±1.53
40.	Foot Tubercles	35 days	29.87 ±1.26
41.	Fore Limbs Visible	38 days	29.64 ±1.72
42.	Fore Limbs Emerge	40 days	28.78 ±0.88
43.	Tail Atrophies	43 days	23.28 ±1.74
44.	Tail Greatly Reduced	45 days	15.53 ±2.04
45.	Tail Stub	46 days	12.16 ±0.64
46.	Metamorphosis Complete	47 days	9.07 ±0.47

Table 5: Food items of tadpoles of *Kaloula pulchra* + = Occurrence - = Non occurrence

Food items		Operculum Complete (Stage 25)	Hind Limb Bud Development (Stages 26-30)	Toe Development (Stages 31-41)
Bacillariophyceae	Diatoma	+	+	+
	Navicula	+	+	+
	Pinnularia	-	+	+
	Stauroneis	+	+	+
	Tabellaria	+	+	+
Chlorophyceae	Closterium	-	+	+
	Cosmarium	+	+	+
	Crucigenia	+	+	+
	Snedesmus	+	+	+
Cyanophyceae	Spirogyra	+	+	+
	Anabaena	+	+	+
	Nostoc	+	+	+
	Oscillatoria	+	+	+
Cryptophyceae	Arcella	+	+	+
	Cryptomonas	+	+	+
Euglenophyceae	Euglena	-	+	+
	Phacus	-	-	+
Zooplanktons	Lecane	-	+	+
	Paramecium	-	-	+

Table 6: Length of intestine of *Kaloula pulchra* at different developmental stages

Gosner Stage	Total length (in mm)	Gut length (in mm)	Gosner Stage	Total length (in mm)	Gut length (in mm)
25	9.56	21.64	36	28.24	37.45
26	14.04	24.23	37	28.37	42.80
27	17.83	25.52	38	29.21	54.32
28	20.85	26.48	39	29.40	57.85
29	21.26	27.42	40	29.92	62.75
30	21.30	28.36	41	29.78	46.72
31	22.24	29.84	42	28.85	30.52
32	24.12	31.25	43	23.36	29.87
33	25.20	32.91	44	15.76	21.45
34	25.51	34.27	45	12.04	20.26
35	26.28	35.65	46	9.12	12.82

Table 7: Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Kaloula pulchra*

Correlations				
Pearson Correlation	Stage	1	0.945**	0.921**
	Total length	0.945**	0.805**	1
	Gut length	0.921**	0.805**	1
Sig. (2-tailed)	Stage	.0	0.000	0.000
	Total length	0.000		0.000
	Gut length	0.000	0.000	
N	Stage	16	16	16
	Total length	16	16	16
	Gut length	16	16	16

**Correlation is significant at the 0.01 level (2-tailed).

Table 8: Correlations between developmental stages from stage 40 to stage 46 along with total lengths and gut lengths of *Kaloula pulchra*.

Correlations				
Pearson Correlation	Stage	1	-0.965**	-0.947**
	Total length	-0.965**	1	0.844*
	Gut length	-0.947**	0.844*	1
Sig. (2-tailed)	Stage	.	0.000	0.001.
	Total length	0.000	-	0.017
	Gut length	0.001	0.017	-
N	Stage	7	7	7
	Total length	7	7	7
	Gut length	7	7	7

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Reproductive Behaviour of *Raorchestes shillongensis* (Pillai and Chanda, 1973) from Meghalaya, Northeastern India

Abstract

We studied reproductive behavior of *Raorchestes shillongensis*, a poorly known endemic bush frog of Northeast India. During the study period, behavioural repertoire previously unreported from other bush frog species of India were observed. We also describe amplexus, spawning and male advertisement call of the species and provide a comparative account of reproductive mode of other bush frogs from South Asian region. Our study fills the gap of knowledge on reproductive biology of bush frogs of Northeast India.

Introduction

Among the tetrapod vertebrates, amphibians exhibit highest diversity of reproductive modes (Haddad and Prado, 2005; Wells, 2007; Crump, 2015; Gaitonde et al. 2016). There are over forty recognized reproductive modes in amphibians (Gururaja et al. 2014; Iskandar et al. 2014; Seshadri et al. 2014; Willaert et al. 2016). Among anurans, the Rhacophorid group exhibit diverse reproductive modes, ranging from foam nests and free-feeding tadpoles to direct development (Brown & Alcalá 1982, 1994; Grosjean et al. 2008). Terrestrial direct development in Bush frogs (*Philautus*, *Pseudophilautus* and *Raorchestes*) involves eggs hatching into tiny froglets avoiding the free swimming tadpole stage (Bossuyt & Dubois, 2001; Bahir et al. 2005; Gururaja and Ramachandra, 2006; Grosjean et al. 2008; Li et al. 2009; Biju et al. 2010). Studies on the reproductive behavior of amphibian species in India has been scanty and data on breeding behavior are available for roughly 7-8 percent of the total amphibian species of the country. Studies on the breeding biology of amphibians is of crucial importance for the successful conservation the species along with their habitats (Gaitonde et al. 2016), and more so for endemic and poorly known species like *Raorchestes shillongensis*.

Meghalaya state in Northeastern India is a part of Indo-Burma global biodiversity hotspot (Mittermeier et al. 2004). The vegetation of the area ranges from tropical evergreen, tropical semi evergreen, tropical moist, riverine grassland, subtropical pine forest and temperate forest (Haridasan and Rao, 1985 & 1987).

Atleast 70 species of amphibians are recorded from Meghalaya state of which about 30% are endemic to the state (Ahmed et al. 2009; Mahony et al. 2013). 10 species of Bush frogs of genera *Raorchestes* and *Philautus* are known from Northeastern India. Meghalaya is home to 6 species (Frost, 2017).

In this communication, we described reproductive behaviour of critically endangered X- mas Bush frog, *Raorchestes shillongensis* which is endemic to Northeastern India.

Key words:
Raorchestes shillongensis,
endemic,
reproductive
behaviour,
Northeast India.

Raorchestes shillongensis male calling.
Photo Credit: Abhijit Das

Bitupan Boruah¹, Uttam Saikia² & Abhijit Das^{*1}

¹ Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand-248007

² North Eastern Regional Centre, Zoological Survey of India, Risa Colony, Shillong-793003

Corresponding author e-mail: abhijit@wii.gov.in



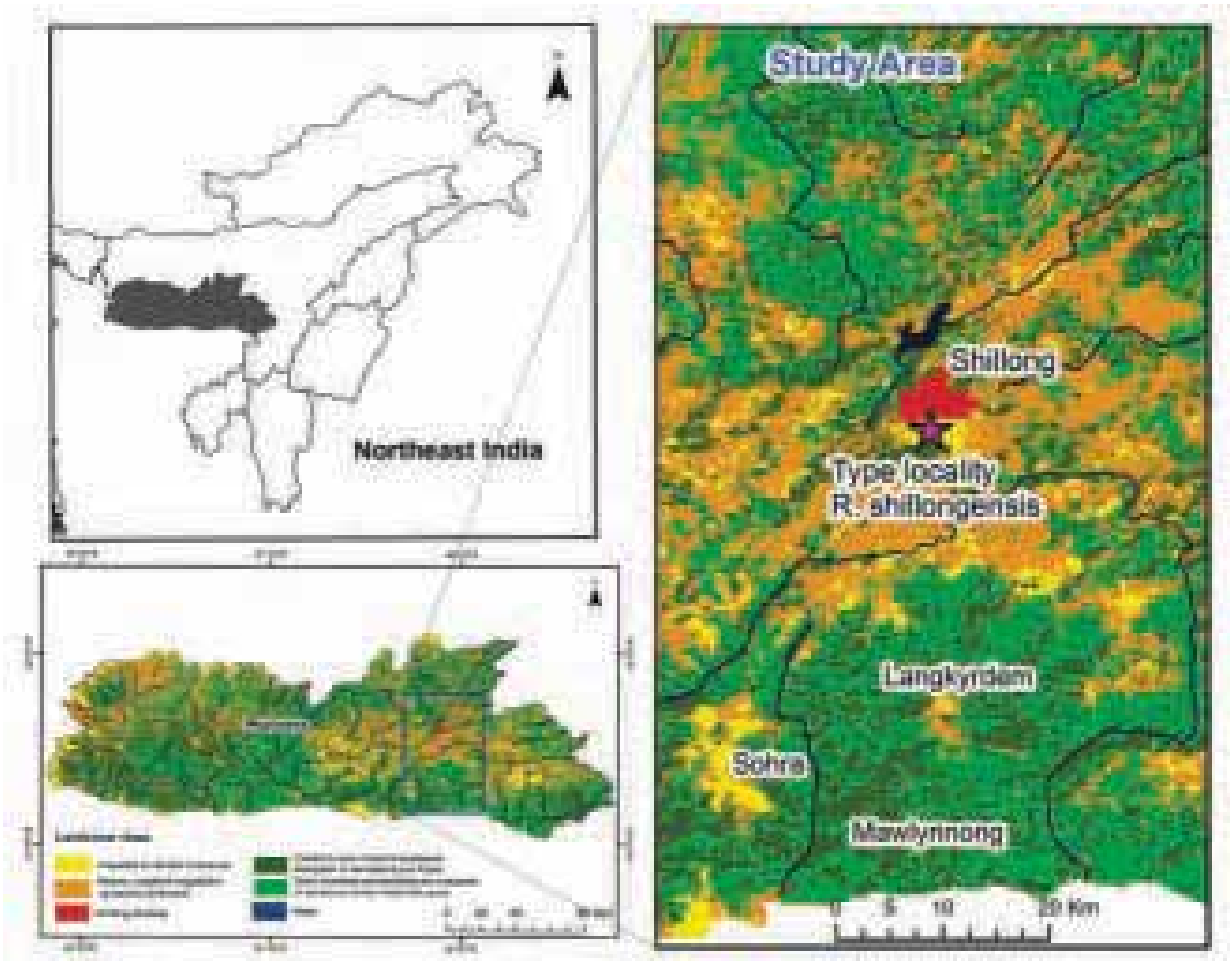
Materials and Methods

Study Area

The Xmas Bush frog *Raorchestes shillongensis* was described from Malki forest (25°33'45''N and 91°53'19''E) within Shillong city limit (Figure 1) in Meghalaya (Pillai and Chanda, 1973). Malki forest is a strip of subtropical wet hill forest (Champion & Seth 1968), dominated by *Pinus kesiya* and broadleaf trees (Mahony et al. 2013). Moss, ferns, tall grasses and other

shrubs cover ground floor of the forest. Forest edges mostly covered by *Eupatorium*, *Melastoma* etc. Malki forest is surrounded by thickly populated human dominated landscape. Some protected and reserve forests viz. Upper Shillong, Riat-Laban, Shyrwart, Laitkor, Mawpat, Mawlai and Riat Khawan are located at the fringe areas of the Malki forest.

Figure 1: Map showing the study area.



Field survey

Field study was conducted between May and August 2016 in the East Khasi Hills district of Meghalaya, Northeast India. During this period, selected forest areas across the district were actively surveyed to record the presence of the species. Night surveys were primarily aided by its distinctive call. The frogs begin to call with the first showers during April end. Day time

survey was also conducted to locate and determine the egg clutch size. Individuals

period, sequences of breeding activities were observed on five occasions within these two study sites (29.05.2016/ 02.06.2016/ 06.06.2016/ 25.06.2016/ 07.07.2016). Observations were made from approximately 1m distance using a neutral white light source and sometime redlight. Time period of each event was recorded in minutes and hours using a stopwatch. Within 10 minutes after egg laying, clutch size, egg diameter, size (SVL) of the amplexant pair was recorded and evaluated the association between female's body size and clutch size and egg size. Development of the eggs was continuously observed for two clutches in natural habitat. Besides, two egg clutches were carried to the field station and kept in nature like condition (with a maximum 1°C temperature difference from the outside) to evaluate the percentage of successful hatchlings. Froglets after hatching were released back to their natural habitat. Basic statistical analysis was performed in MS excel spreadsheet.

Call record and analysis

A digital recorder (Sony IC recorder 7.4.0) was used to record call on the 30th August at 19.41 hrs. Recorded calls were visualized and call characters were obtained using Raven Pro Ver. 1.5 (Charif, Waack & Strickman, 2010). We measured a total of five temporal properties that included call group duration, inter call group interval, intra call group interval, call duration and

call rate of a call bout comprising of five call groups. One spectral property, i.e., peak frequency, was also measured over the entire series of calls. Terminologies and graphical representation of the call properties follow Bee et al. (2013a) and Bee et al. (2013b).

Results

Male vocalization: Calling males were recorded from the onset of raining in April end and the call activities decreases towards August. Calling males come out at just beginning of dusk (18:30 hr) and calling is more frequent up to 24:00 hr. Calling of males also can be heard from the bushy thickets on a rainy day. Advertisement calls delivered in call groups (Figure 2) consists of two to multiple (Tick tick tick ...) calls. Duration of the call bout analysed was 22.62s and number of call group was five with call numbers varying 3-5. Duration of call group was $0.72 \pm 0.21s$ ($n=5$). Duration of call group varies with the number of calls. Inter call group interval was $4.76 \pm 0.76s$ ($n=4$). Intra call group interval was $0.19 \pm 0.02s$ ($n=14$). Call had a single pulse (non-pulsatile) for a short moment of $0.05 \pm 0.01s$ ($n=19$). Overall peak frequency of the calls was 3.62 kHz.

Sexual size dimorphism: Male individuals of *R. shillongensis* has body size (snout-vent length, SVL) of 17.7 ± 1.51 mm, $n=54$; females had SVL of 18.9 ± 1.43 mm, $n=14$. Body weight of the male (0.54 ± 0.15 g, $n=54$) is less than that of female (0.71

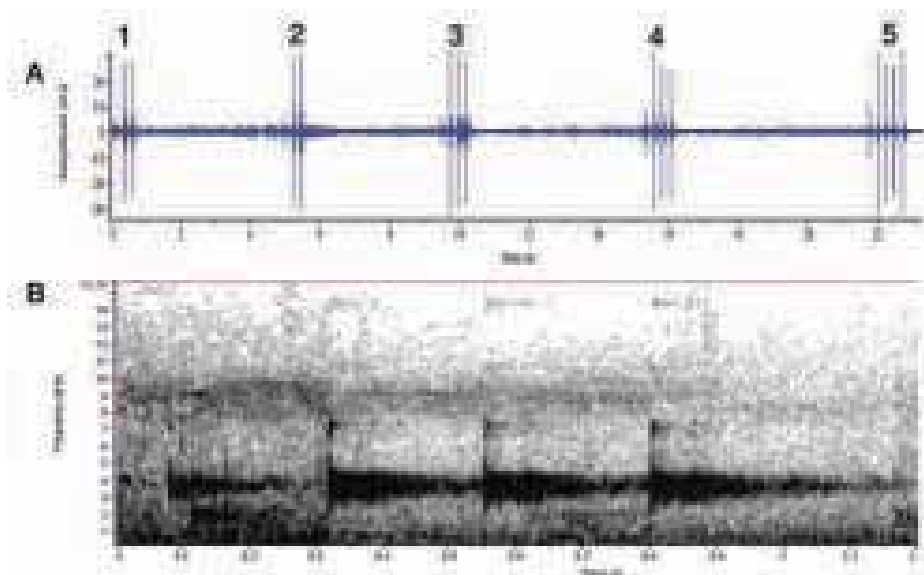


Figure 2: Advertisement call of *R. shillongensis* at ambient air temperature 24.1°C. A. A call bout comprising of five call groups (digits indicated number of calls in the respective call groups). B. Spectrogram of 4th call group with four calls depicted in A.

± 0.19 g, $n=14$).

Reproductive strategy: Description of the reproductive behaviour presented is based on five observations. Males start calling intensively after the dusk (18:00-18:30hr). Calling activity declines towards late night (after 24:00 hr). Males perch and call from branches or leaves of shrubs. Male's perch height is 69.2 ± 38.73 cm ($n=73$) and female's perch height is 52.53 ± 42.02 cm ($n=15$) above ground (Figure 3).

Amplexus: Males start calling extensively after the dusk (about 18:00 hr). The gravid female (with large ripe ova externally visible from lateral and ventral side) moves slowly following the calling male. When the female reaches very close to the calling male, the male quickly grasps the female and engages in axillary amplexus (Figure 4). Amplexus starts on an arboreal situation and lasts for 9-11 hours. We did not observe any competition among males during their amplexus. Amplexus were observed at late evening between 21:05 to 23:40 hr. Once the pair comes into physical contact, the male generally ceases calling, but in one case amplexant male continued calling with low

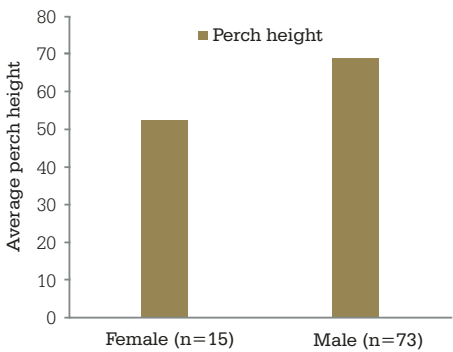


Figure 3: Comparison of perch height between male and female individuals of *R. shillongensis*.

pitch for initial four minutes. Between 21:05 hr to 04:00 hr, the amplexus pair remains in the arboreal habitat for 7.05 ± 1.56 hours ($n=5$). Between 04:00-05:45 hr, the amplexant individuals (the male piggybacking the female) descends to the ground and moves in search of a suitable place to lay egg. After 1.73 ± 0.31 hours ($n=5$) of descending to ground, they go inside leaf litters. By the time, the body colour of the amplexant female becomes dark and camouflages with the moist soil. This change is prominent in female than

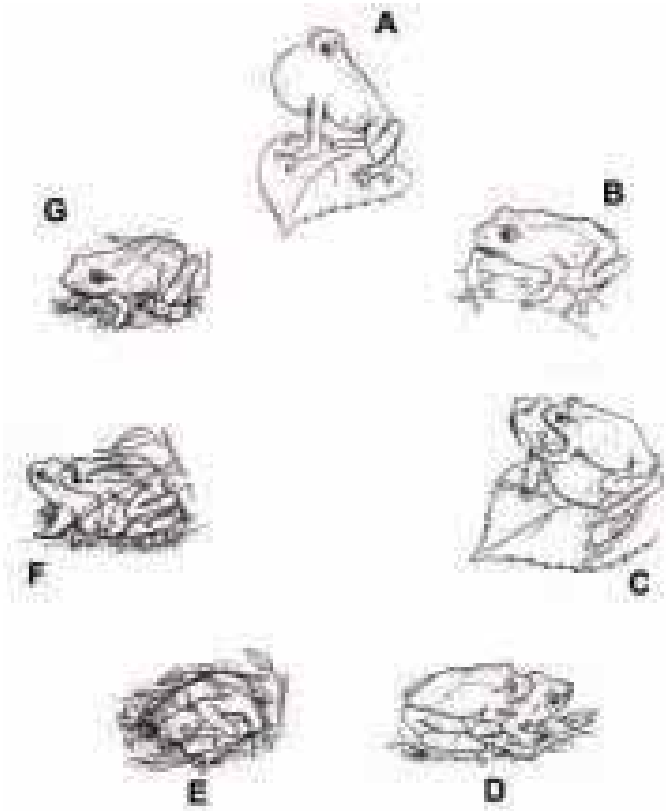


Figure 4: Schematic representation of various sequences of mating behavior in *R. shillongensis*. A- A calling male from its perch; B- A gravid female slowly approaching towards the calling male; C- Male & female in axial amplexus; D- Amplexant pair on ground in search of a suitable place; E- Amplexant pair entering under leaf litters for egg lay; F- Egg laying and fertilization under leaf litters; G- Mixing of fertilized eggs with moist soil by the female after the male dislodged.

male. When the female finds a suitable place (within approximately 30cm distance from the plant from where they descend) in the moist soil she stops moving. Then she rotates in semicircle in either side with the amplexant male. Besides the semicircular movement, the female was also observed to bob her head although causation of this behavior could not be ascertained. In a few instances, the amplexant pair fell down while moving in arboreal habitat and dislodging of the amplexant male; but they resumed amplexus quickly. Details of the observations are given in the Table 2.

Oviposition and egg clutch: Female lay 8-17 eggs on moist soil under leaf litters, early in the morning between 7:30-10:10 hr. Eggs are rounded, unpigmented and with a transparent jelly coat (Figure 7). Eggs are laid in clump and diameter of eggs ranges between 2.42-5.2mm ($n=47$). At the time of egg laying the female slightly lifts her body and male fertilizes the eggs. As soon as fertilization completed the male dislodges and leaves the place. The male cleans its body with limbs while leaving the place. As the male leaves the place, female starts dragging moist soil from her surroundings,

rotating on either side and keeping her cloaca near the laid eggs. She uses both hands to gather soil alternatively with a very short interval. After that, she mixes the egg mass with gathered soil particles using her hind limbs alternatively. During this process, eggs do not get separated. Time taken by the female to mix the eggs with soil is 16 ± 5.15 min (n=5). After completion of the process of mixing the eggs with soil, the female cleans her body and leaves the

place. In one case, the female stayed near the eggs for about 4 minutes.

Regression analysis showed that the female SVL is strongly correlated with clutch size ($R^2 = 0.81$, n=5) but correlation with egg size is not very significant ($R^2 = 0.49$, n=4) (**Figure 6**).

As in other *Raorchestes* species, development in *R. shillongensis* is direct; i.e. without free swimming tadpole stage



Figure 5 : *R. shillongensis*. A. A calling male; B. An amplexant pair.

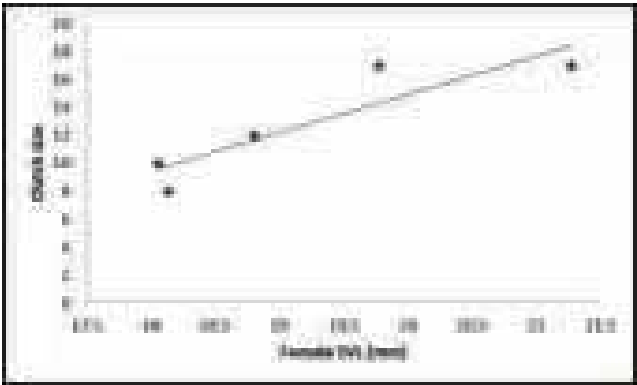


Figure 6 : A. female SVL shows a strong positive correlation with number of eggs ($R^2 = 0.81$) and B. egg size ($R^2 = 0.49$).

(**Figure: 7**). Incubation or developing period is 30.5 ± 0.71 days (n=2) (details of egg development is provided in **Table 1**). Rate of successful hatchlings is 100% (n=2). Body colour of the froglet is brown and slightly blackish towards lateral side of the belly or brown with dark -(-) shape mark on back as in adult.

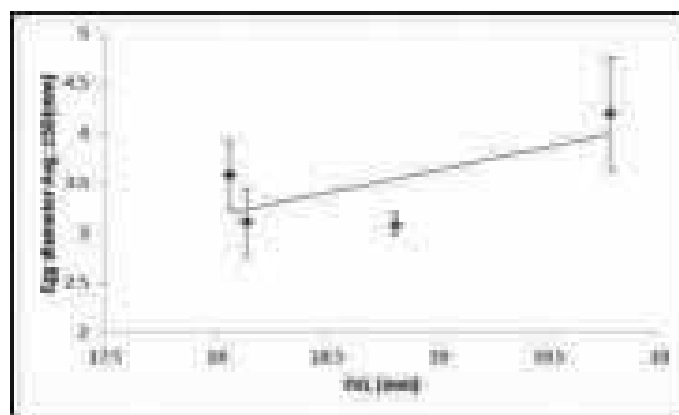
Discussion

The present study is a first ever documentation of breeding behavior of a



6A

bush frog from Northeast India. Breeding mode of *R. shillongensis* belongs to Type 17 as delineated in Duellman and Trueb (1994), i.e. direct development on ground. Clutch size of *R. shillongensis* is smaller (except *R. chalazodes* and *R. ochlandrae*) but developmental duration is longer than other known Indian bush frogs (Table 3). This may be attributed to the colder and more moist conditions the region. The Amplexus mode, egg colouration and size is comparable to other Bush frogs. Reproductive mode *R. shillongensis* is similar (mode 17) with *R. tinniensi*, *R. graminirupes* and *R. resplendens*. But, *R. resplendens* lays eggs under moss covered forest floor, deep inside the base of bamboo clumps (Biju et al. 2010), *R. graminirupes* lays on ground, grass clump, rocky crevices, *R. tinniensi* lays in deep hole on ground (Gururaja and Ramachandra, 2006), while *R. shillongensis* lays eggs on moist soil under leaf litters. However, mixing of eggs with moist soil has not been previously observed in any bush frogs of India. In *P. variabilis*, (confined to Sri Lanka) the female guarded the eggs and chase the males (Kanamadi et al. 1996). However, we did not observe such parental care in *R. shillongensis* like in *R. chalazodes* and *R. ochlandrae* as they stay with the eggs until hatched. Other bush frogs in India like *Raorchestes gladulosus*, *R. bombayensis*, *R. nerostagona* and *R. bobingeri* deposits eggs above the ground on arboreal habitats (mode 20). Breeding behaviour of *R. shillongensis* shows some similarity with ground nesting *Pseudophilautus* spp. of Sri Lanka as they mix the eggs with soil, probably for better distribution of sperms (Bahir et al. 2005). However, *R. shillongensis* do not excavate soil to egg lay and egg separation does not occur during mixing with soil as in Sri Lankan *Pseudophilautus* (Bahir et al. 2005). Clutch size of *R. shillongensis* is relatively smaller than other bush frogs except *R. chalazodes*, *R. ochlandrae* and Sri Lankan bush frog *Pseudophilautus regius*. The change in body colour of *R. shillongensis* to camouflage with soil during egg laying is not reported in any Indian bush frogs except Sri Lankan ground nesting bush frogs (Bahir et al. 2005). The colour change of ground-nesting bush frogs (*Pseudophilautus*) during egg laying is probably to reduce the predation risk, especially these frogs often



6B



Figure 7: Developing embryo of *R. shillongensis*.

1. Stage 2-3,
2. Stage 5-6,
3. & 4. Stage 10
5. Stage 14,
6. A newly hatched froglet.

nest in daytime (Bahir et al. 2005).

Despite the smaller distribution range of *R. shillongensis* (approximately 530 sq. km) in the East Khasi Hills, it is relatively abundant in the backyards and forest edges especially around the Shillong city. During the study period, we observed that the habitat of the Malki forest and adjacent areas are rapidly degrading due to various anthropogenic activities like fire wood collection, intentional forest fire, excessive use of detergent for cloth washing in the forest streams (Mahony et al. 2013), garbage dumping by the local people as well as by the tourist etc. Besides the above factors, use of chemicals for cultivation in the surroundings of Malki and Upper Shillong areas could be detrimental to the long term

survival of this endemic bush frog.

Shillong Plateau is well known for high endemicity of amphibian species, but government-protected forests of the East Khasi Hills district (2,752 km²) represent only 18.65 km² in seven locations, the largest being the Upper Shillong Protected Forest with only 7.66 km² area (Mahony et al. 2013). Deteriorating habitat quality, unregulated mining in protected and unprotected areas in the state (Gilbert, 2012), may have considerable affect in the survival of amphibian fauna of the region (Mahony et al. 2013). Therefore, conservation of the last remaining forest patches is of urgent priority, which harbor many threatened and endemic species of the region.

Acknowledgement

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Raorchestes spp. of north-east India largely represent cryptic diversity.
Photo Credit: Abhijit Das



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Table 1: Developmental stages of embryos of *R. shillongensis* (Based on clutch laid on 08. 06. 2016). Stages are in comparison to *Pseudophilautus viridis* from Sri Lanka (Bahir et al., 2005).

Date	Days from the date of laid	Stage	Characteristics
19.06.2016	12	Stage 2-3	Limb buds clearly visible, unpigmented subderal eyes, tail elingated.
22.06.2016	15	Stage 4	Eyes large and black, pigment on dorsal side of the yolk
24.06.2016	17	Stage 5-6	Toe demarcation initiated, eye pupil visible.
28.06.2016	21	Stage 10	Limbs fully developed, toes and fingers visible, pigmentation covers limbs and spread towards lateral side of the yolk.
05.07.2016	28	Stage 14	Tail almost absent, little amount of yolk present, looks like adult.

Table 2: Ecological parameters and sequences of breeding behaviour of *R. shillongensis* during the study period.

Date	Temp/ Humidity (°C/%)	Perch height (M/F) (cm)	SVL (M/F) (mm)	Perching distance between M & F (cm)	Amplexus start time
29.05.16	17.1/ 93	51/120	17.56/18.06	100	21:05 hr
02.06.16	17.8/ 92	63/40	16.37/18.81	23	21:50 hr
07.06.16	18.3/89	70 (same leaf)	18.23/21.28	very close	21:15 hr
25.06.16	20.8/92	52/78	17.73/18.14	30	22:25 hr
06.07.16	21.6/82	77/50	16.85/19.78	60	23:40 hr

Table 3: A comparative chart of different reproductive modes in Bush frogs of India and Sri Lanka.

Species name	SVL (mm)	Clutch size	Days to hatch	Reproductive Mode
<i>Philautus cf. leucorhinus</i>	♀: 33.7♂: 28.9	51	19	20 (Direct development and arboreal)
<i>P. glandulosus</i>	♀: 24.5-26♂: 20-22.9	41	28	20 (Direct development and arboreal)
<i>P. variabilis</i>	♀ & ♂: 30.0 ±4.5	54-62	-	20 (Direct development and arboreal)
<i>P. nerostagona</i>	♀: no report ♂: 30.1-34	41	20	20 (Direct development and arboreal)
<i>P. tinniens</i>	♀: 25♂: no report	-	-	17 (Direct development on ground)
<i>R. bombayensis</i>	-	26-27	-	20 (Direct development and arboreal)
<i>P. bobingeri</i>	♀: 23.5-26♂: 21.3-24.8	24	18	20 (Direct development and arboreal)
<i>P. graminirupes</i>	♀: 27.3-29.4♂: 21.4-22.6	30-38	24	17 (Direct development on ground)
<i>R. resplendens</i>	♀: 25.2-28.3♂: 22.7-24.5	18-28	-	17 (Direct development on ground)
<i>R. ochlandrae</i>	♀: 23.3♂: 24.0±1.38 (5)	6	-	20 (Direct development and arboreal)
<i>R. chalazodes</i>	♀: 25.2♂:23.7 ±2.66 (3)	5-8	-	20 (Direct development and arboreal)
16 species of <i>Pseudophilautus</i> #	-	6-155	24-68	17 (Direct development on ground)
<i>Pseudophilautus femoralis</i>	-	7-22	37-49	20 (Direct development and arboreal)
<i>P. regius</i>	-	17	-	17 (Direct development on ground)
<i>R. shillongensis</i>	♀: 18.47 ±1.6 (12) ♂:16.5 ±1.31 (25)	8-17	30-31 (2)	17 (Direct development on ground)

Time of descending to ground	Time of entering under leaf litters	Time of egg laying completion	Male dislodge time	Time of completion of egg mixing process	Female Leaving time	Clutch size	L/B of clutch (mm)
05:00 hr	07:05 hr	08:00 hr	08:02 hr	08:20 hr	08:23 hr	10	12.58/10.23
05:45 hr	07:35 hr	07:55 hr	07:57 hr	08:10 hr	08:13 hr	12	19.44/16.67
05:15 hr	06:55 hr	07:30 hr	07:31 hr	07:45 hr	07:50 hr	17	19.01/11.51
05:30 hr	07:20 hr	08:10 hr	08:13 hr	08:37 hr	09:05 hr	8	14.51/9.04
04:00 hr	05:15 hr	10:10 hr	10:14 hr	10:25 hr	10:27 hr	17	15.66/13.72

Egg Laying Habitat	Parental Care	Egg diameter (mm)	Study Locality	Data source
Above ground, (10cm) on wet leaves, between rocks	Pair separates after spawning	3.5 ±0.16 (51)	Karnataka	Gururaja & Ramachandra, 2006
Above ground (1.5-3m) on wet leaves	Pair separates after spawning	4.4 ±0.2 (48)	Waynaad, Kerala	Biju, 2003
Above ground	Eggs beneath abdomen of female, chasing intruding males	4.1 ±0.2 (30)	Karnataka	Kanamadi et al., 1996
Above ground (10m) in tree hole (10cm deep)	-	4.5 ±0.3 (41)	Waynaad, Kerala	Biju & Bossuyt, 2005
On ground	-	-	Nilgiri hills, Tamil Nadu	Bossuyt & Dubois, 2001; Dubois, 1986
Above ground on wet leaves	-	-	Karnataka	Bossuyt et al. , 2001
Above ground (4m) on Acacia tree	-	3.9 ±0.4 (24)	Ponmudi hills, Kerala	Biju & Bossuyt, 2005
On ground, grass clump, rocky crevice	-	4.9 ±0.5 (38)	Ponmudi hills, Kerala	Daniels, 2005
under moss cover forest floor	Leaves after spawning	4.1 ±0.4 (24)	Eravikulam National Park, Western Ghats	Biju et al., 2010
Above ground, inside bamboo internode	Male attends the eggs until hatch	4.94 ± 0.06 (developing embryo)	Kerala	Gururaja et al., 2007
Above ground (25cm), inside bamboo internode	Male attends the eggs until hatch	5.73 ±0.66 (28)	Kalakad Mundanthurai Tiger Reserve, Western Ghats	Seshadri et al., 2014
In soil cavity (1.5-50 cm3)	Male voluntarily departures, female abandons after concealing the eggs	3.7-5.7	Sri Lanka	Bahir et al., 2005
on underside of leaf above ground (0.3-2m)	after male dislodged female sits on the eggs for 1-3 hrs	-	Sri Lanka	Bahir et al., 2005
Soil cavity	Female burrows the eggs and leaves	3.1 (17)	Sri Lanka	Karunarathna & Amarasinghe, 2007
On moist soil under leaf litters	Male leaves after fertilization and female leaves after egg mixing with soil	3.59 ±0.63 (47)	Malki Forest Shillong, Meghalaya	Present study





SECTION

III

ECOLOGY AND CONSERVATION

Heavy metal pollution of aquatic systems and its possible impact on anuran tadpoles: a study in Barak Valley, Assam, India

Abstract

In the present study, investigations were made on the accumulation of heavy metals- copper (Cu) and lead (Pb) in water, sediment and tadpoles inhabiting the water bodies of Barak Valley, Assam. Tadpoles of *Microhyla ornata*, *Duttaphrynus melanostictus*, *Hoplobatrachus tigerinus* and *Polypedates teraiensis* were collected from eight different sites and heavy metal concentrations of Copper and Lead were determined in the whole body of tadpoles. Heavy metal concentrations in water and sediment collected from the habitat of tadpoles were also analyzed. The results revealed that the copper concentration in water samples was within the maximum permissible limit of WHO (2 mg L⁻¹), but the concentration of lead in water samples increased beyond the permissible limit of WHO (0.01 mg L⁻¹). The result of heavy metal analysis in tadpoles also revealed that tadpoles of *Microhyla ornata* collected from an industrial area showed highest concentrations of copper and lead whereas tadpoles of *Duttaphrynus melanostictus* collected from AUS campus showed the lowest concentration of metals. From the present study it can be concluded that the tadpoles can be considered as a good bio-indicators of metals contamination in streams, wetlands and other aquatic bodies.

Introduction

Amphibians are good bio- indicators of ecosystem health and can indicate pollution level. (Kelepertzis et al., 2012, Loumbourdis & Wray, 1998). According to IUCN Red list nearly 41% of amphibian species are globally threatened (Hoffman, 2010). The dramatic decline in amphibian population worldwide is considered to be one of most critical threats to global biodiversity and measures of conservation for amphibians need to be designed which include creating nature reserves and national parks, monitoring of habitats as well as carrying out public awareness and educational outreach programmes. Various studies have opined that more than 76% of the world's organisms will face extinction in the next 300 years plunging the world into another mass extinction (Barnosky et al. 2011).

Amphibians are affected by environmental pollution due to their susceptibility to chemicals and high sensitivity to perturbations in the environment. They are

well known for accumulating metals during their freshwater cycles (Loumbourdis & Wray, 1998). Bioaccumulation is the building up of a chemical up to a toxic level in the body of an organism. It is the net accumulation of a substance by an organism as a result of uptake directly from all environmental sources and from all routes of exposure (ASTM, 1998). As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in

Key words:

Anuran tadpoles,
Heavy metal,
pollution, Copper,
Lead, South
Assam.

Microhyla sp. tadpoles
Photo Credit: Vishal Prasad

Pammi Singh¹, Mithra Dey^{*1} & Sunkam Narayana Ramanujam²

¹Department of Ecology and Environmental Science, Assam University, Silchar-788011, Assam, India

²Department of Zoology, North Eastern Hill University, Shillong 793022, Meghalaya, India.1

*Corresponding author e-mail: mithradey@gmail.com



water, sediment and aquatic animals causing heavy metal pollution of water bodies (Mallick et al. 2010). Larval amphibians accumulate metals more readily than adults, possibly due to their semi permeable and highly vascularised skin which allows cutaneous respiration and high accumulation of environmental pollutants in the tissue from water and soil directly (Hall & Muller, 1984). In addition tadpoles during the development period are microphagous in feeding habit and ingest sediment in which heavy metals accumulate (Hopkins & Rowe, 2010).

One important reason that has been implicated as a cause of decline of amphibian and fish population in aquatic systems is contamination with heavy metals which is mainly from anthropogenic sources (Greig et al. 2010; Hopkins & Rowe, 2010). The anthropogenic sources include industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbor activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz, 2009). Heavy metals are also known as trace elements because they occur in minute concentrations in biological systems. Some metals are potentially toxic (As, Cd, Pb, Hg, etc.), while others are probably essential (Ni, V, Co), and many are essential (Cu, Zn, Fe, Mn) (Biswas et al. 2012). These essential metals can also produce toxic effects when the metal intake is excessively elevated (Tekin- Ozan, 2008). The heavy metals are hazardous because of their toxicity, persistence, and bio accumulation in the food chains. Frogs are opportunistic breeders and breed in wide variety of aquatic systems like ephemeral pools, small and large ponds, streams, ditch, drains, manmade tanks etc. The present study was conducted in Barak Valley region of Assam, India and heavy metal accumulation of Cu and Pb was analysed in water, sediment and whole body of anuran tadpoles collected from different types of habitat. Considering the body size of the tadpoles, heavy metal analysis was done in the whole body of the tadpoles (Gosner stage 26-35, tadpole size range: 10.3- 27.1 mm). Tadpoles of *Microhyla ornata* (MO), *Duttaphrynus melanostictus* (DM), *Hoplobatrachus tigerinus* (HT) and

Polypedates teraiensis (PT) were collected from eight different sites and heavy metal concentrations were determined in the whole body of tadpoles. The heavy metal concentrations of Copper (Cu) and Lead (Pb) was analyzed. Heavy metal concentrations of Cu and Pb in water and sediment collected from the habitat of tadpoles were also analyzed.

Materials and Methods

Barak Valley is situated in the southern part of the Assam, between 24°8'N and 25°N latitudes and 92°15'E and 93°15'E longitudes. The region abounds in wetlands, streams, pools, marshes, ponds etc. of various shapes, sizes and have small hillocks. The valley has urban areas, brick kilns, industrial area like Cachar Paper Mill at Panchgram situated at a distance of 25 Km from Silchar town and large number of tea gardens. The study was carried out in different selected habitats of Barak Valley which included tea estates, urban and industrial area and brick kilns. For physicochemical analysis and determination of Cu and Pb in water, three replicates of water samples were collected from the sites where tadpoles were present. Physicochemical parameters like pH, electrical conductivity (EC), dissolved oxygen (DO), free carbon dioxide (FCO₂) and total alkalinity (TA) were analyzed using standard methods (APHA, 2005; Trivedy & Goel, 1984). Tadpoles were collected by dip net and were washed properly with double distilled water in the laboratory. Drying of the whole body of tadpole was done until a constant weight was obtained. Digestion of all tadpole samples was done according to FAO/SIDA (Swedish International Development Authority Cooperative Programme, 1983). To each sample (0.1 g), 10 ml of perchloric acid: conc. HNO₃ (3:2 v/v) was added and the mixture was heated at 60°C until a clear solution was formed. The resulting solutions were cooled, and the volumes were made up to 50 ml using double distilled water. The samples were then stored in plastic bottles till analysis to determine the amount of heavy metal bioaccumulated (Ezemonye & Enuneku, 2012). The water collected in sampling bottles were preconditioned with dilute nitric acid (HNO₃) and later rinsed thoroughly with double distilled water.

Precleaned polyethylene sampling bottles were immersed about 10 cm below the water surface and 1 liter of the water sample was taken. Samples were acidified with concentrated nitric acid (HNO₃) for preservation. The samples were filtered through Whatman filter paper No. 1 and kept in refrigerator until analysis. The sediment samples were oven dried at 45°C followed by grinding and sieving using <2 mm sieve, 5 gm of dry sample was poured into a beaker and mixed with 2 ml of aqua regia (1 conc. HCL : 3 conc. HNO₃). The mixture was digested on a hot plate in open beakers at 95°C for 1 hr and allowed to cool to room temperature. The supernatant was filtered and then diluted to 50 ml using distilled water. The heavy metal concentrations in the digested samples of tadpoles, water and sediment were determined in a Graphite Furnace-Atomic Absorption spectrophotometer (GF-AAS), Model Analytik Jena Vario-6.

Abbreviations used: MO: *Microhyla ornata*, DM: *Duttaphrynus melanostictus*, HT: *Hoplobatrachus tigerinus* and PT: *Polypedates teraiensis*.

Results

Heavy metal concentrations and bioaccumulation in whole body of tadpoles

For heavy metal analysis tadpoles were collected from different habitats of Barak Valley which included tea estates, rural, urban and industrial area and paddy fields during 2013 and 2014. Approximately 50-55 nos. of tadpoles of each species was sacrificed. Table 1 shows the habitats used by anuran tadpoles. Concentrations of two heavy metals-Copper and Lead in the whole body of tadpoles are given in Table 2. Copper concentration in whole body of different tadpole species ranged between $0.411 \pm 0.06 \mu\text{g gm}^{-1}$ to $2.07 \pm 0.01 \mu\text{g gm}^{-1}$. Tadpoles of *Microhyla ornata* collected from Panchgram accumulated the highest concentration of Cu ($2.07 \pm 0.01 \mu\text{g gm}^{-1}$); while *Duttaphrynus melanostictus* collected from Assam University Silchar (AUS) campus showed the lowest values of Cu ($0.411 \pm 0.06 \mu\text{g gm}^{-1}$). The order of Cu concentration was *Microhyla ornata* (Panchgram) > *Microhyla ornata* (Karimganj) > *H. tigerinus* (Karimganj) > *H. tigerinus*

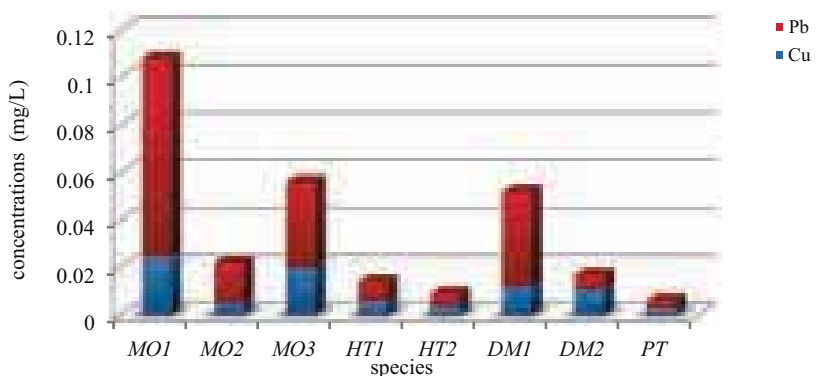


Figure 1: Heavy metal concentrations (mean) in whole body of different species of tadpoles

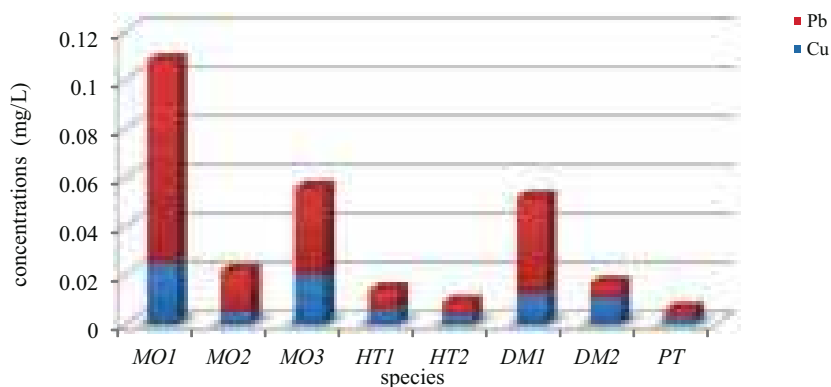


Figure 2: Heavy metal concentrations (mean) in water of different species of tadpole habitats

(Panchgram) > *P. teraiensis* (AUS campus)
> *Microhyla ornata* (Rosekandy) > *D. melanostictus* (Panchgram) > *D. melanostictus* (AUS campus) (Fig. 1).

Copper (Cu) concentration in water (Fig. 2) and sediment (Fig. 3) samples ranged between $0.003 \pm 0.02 \text{ mg L}^{-1}$ to $0.025 \pm 0.04 \text{ mg L}^{-1}$ and $0.634 \pm 0.1 \mu\text{g gm}^{-1}$ to $4.27 \pm 0.06 \mu\text{g gm}^{-1}$ respectively. Water sample collected from the habitat of *Microhyla ornata* (Panchgram) showed the highest concentration of Cu ($0.025 \pm 0.04 \text{ mg L}^{-1}$) while site of *Polypedates teraiensis* (AUS campus) showed the lowest concentration ($0.003 \pm 0.02 \text{ mg L}^{-1}$). Considering sediment samples, habitat of *Duttaphrynus melanostictus* (Panchgram) showed highest conc. of Cu ($4.27 \pm 0.06 \mu\text{g gm}^{-1}$) and lowest was recorded in the habitat of *Polypedates teraiensis* collected from AUS campus ($0.634 \pm 0.1 \mu\text{g gm}^{-1}$).

Lead (Pb) levels in whole body of tadpoles ranged from $0.024 \pm 0.01 \mu\text{g gm}^{-1}$ (*Duttaphrynus melanostictus*, AUS campus) to $2.42 \pm 0.03 \mu\text{g gm}^{-1}$ (*Microhyla ornata*, Panchgram). The order of Pb concentration was *Microhyla ornata* (Panchgram) > *Microhyla ornata* (Karimganj) > *H. tigerinus* (Panchgram) > *D. melanostictus* (Panchgram) > *Microhyla ornata* (Rosekandy) > *H. tigerinus* (Karimganj) > *P. teraiensis* (AUS campus) > *D. melanostictus* (AUS campus) (Fig. 1).

Concentrations of Pb in water (Fig. 2) samples ranged from $0.003 \pm 0.03 \text{ mg L}^{-1}$ (*Polypedates teraiensis*, AUS campus) to $0.083 \pm 0.03 \text{ mg L}^{-1}$ (*Microhyla ornata*, Panchgram). Pb concentration in sediment (Fig. 3) samples ranged from $0.893 \pm 0.21 \mu\text{g gm}^{-1}$ (*Polypedates teraiensis*, AUS campus) to $5.55 \pm 0.08 \mu\text{g gm}^{-1}$ (*Duttaphrynus melanostictus*, Panchgram).

One-way ANOVA was applied to test for differences in trace elements concentration in the whole body of tadpoles among the different sites. All the tadpole species varied significantly ($p < 0.01$) in terms of metal accumulation as revealed by One-way ANOVA (Table 3). One-way ANOVA was also done to determine differences of metal accumulation in different species and the result showed that there was significant variations in terms of Cu ($F = 129.7$, $p < 0.01$) and Pb ($F = 163.1$, $p < 0.01$) accumulation in different species of tadpoles. One-way

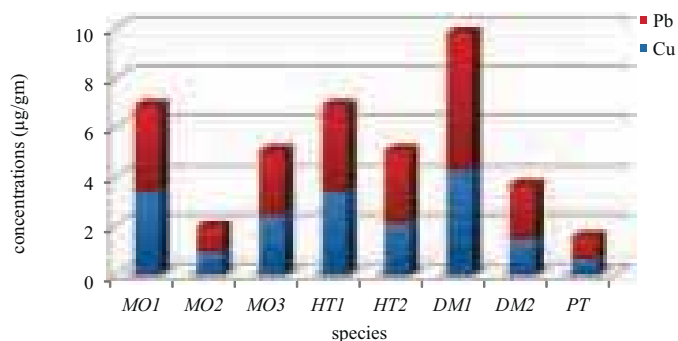


Figure 3: Heavy metal concentrations (mean) in sediment of diff. species of tadpole

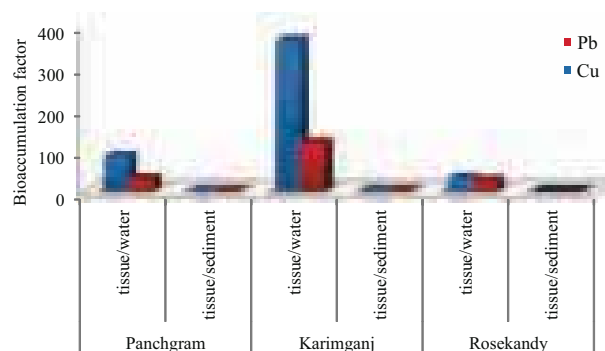


Figure 4: Bioaccumulation factor of heavy metals in whole body of *M. ornata*

ANOVA was done to determine differences of metal accumulation in water and sediment samples at different study sites. The result showed that statistically there was significant variations in metal concentrations in water ($F = 1.9$, $p < 0.05$) and sediment samples ($F = 1.03$, $p < 0.05$) collected from the different sites.

Bioaccumulation factors between metal concentration in tadpoles and water are presented in Fig. 4 to Fig. 7. The highest bioaccumulation factor of copper (Cu) were observed in *Microhyla ornata* from Karimganj and lowest were recorded in *Microhyla ornata* from Rosekandy tea garden. Highest bio accumulation factor for lead (Pb) were observed in *Hoplobatrachus tigerinus* from Panchgram and lowest in *Duttaphrynus melanostictus* from Panchgram.

Bioaccumulation factors between metal concentration in tadpoles and sediment are presented in Fig. 4 to Fig. 7. The highest bioaccumulation factor of copper (Cu) were

observed in *Microhyla ornata* from Karimganj and lowest were recorded in *Duttaphrynus melanostictus* from Panchgram. Highest bio accumulation factor for lead (Pb) were observed in *Microhyla ornata* from Karimganj and lowest were observed in *Duttaphrynus melanostictus* from Assam University campus. The results of Bioaccumulation factor showed that Cu and Pb in tadpoles from water were greater than that from sediment and this implies that the tadpoles bioaccumulated these metals from the water.

Physico-chemical characteristics of water samples

The physico-chemical characteristics of water from different sampling ponds are presented in Table 4. Surface water temperature (WT) ranged between 23 to 25.6°C. Air temperature (AT) ranged between 24.8°C to 28.6°C. pH was low in university campus having value of 5.2 while the highest value of 6.9 was recorded at Panchgram. Ponds in the industrial area had slightly higher pH towards alkalinity. Electrical conductivity showed lowest value at university campus ($40 \mu\text{S cm}^{-1}$) and the highest values were recorded at Panchgram ($95 \mu\text{S cm}^{-1}$, $1.90 \mu\text{S cm}^{-1}$ and $85 \mu\text{S cm}^{-1}$) followed by Rosekandy tea garden ($56 \mu\text{S cm}^{-1}$). Dissolved oxygen level was generally low and remained at the $<5 \text{ mg L}^{-1}$ in all cases and free CO_2 ranged between 8.2 to 13.2 mg L^{-1} . In different study sites total alkalinity ranged between 30 mg L^{-1} to 50 mg L^{-1} . All physiochemical characteristics of water in the tadpole habitats, varied significantly ($p < 0.01$) as revealed by One-Way ANOVA. (Table 5).

Correlation coefficients (r) computed between physico-chemical properties and concentrations of two different heavy metals (Cu and Pb) in water from different sites are presented in Table 6. The concentration of Cu and Pb in water samples was significantly positively correlated with water temperature, pH, electrical conductivity and significantly negatively correlated with dissolved oxygen. Air temperature was insignificantly negatively correlated and free CO_2 and total alkalinity

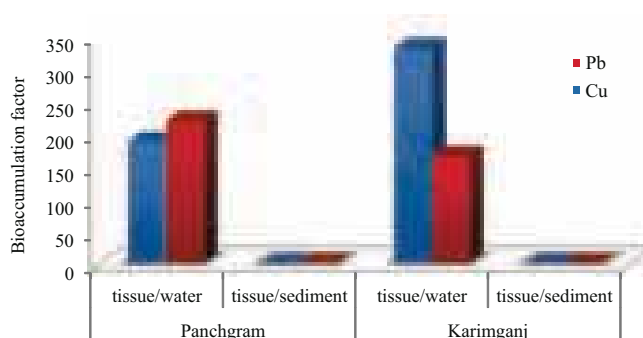


Figure 5: Bioaccumulation factor of heavy metals in whole body of *H. tigerinus*

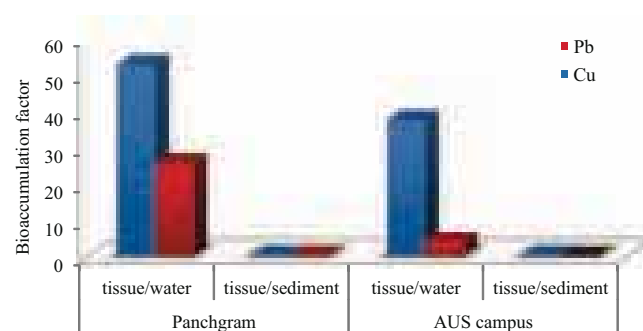


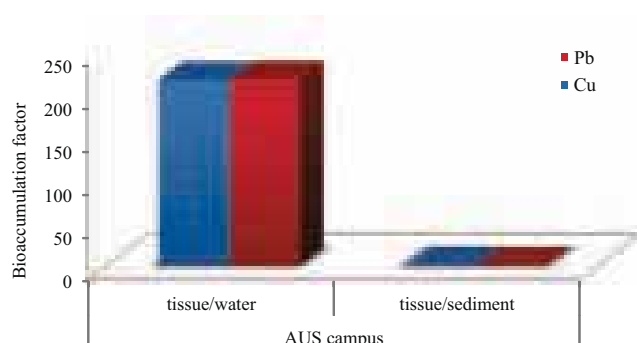
Figure 6: Bioaccumulation factor of heavy metals in whole body of *D. melanostictus*

were insignificantly positively correlated with the concentrations of copper and lead.

Discussion

Different species of tadpoles exhibit different metals accumulation rates which may be due to differences in their physiological roles. Lead concentrations were found to be highest in all the samples (whole body, sediment and water) when compared to copper. The samples collected from industrial area showed the highest concentration of lead. Urban and industrial sewage may be the source of lead in the area. Karasovet al. (2005) reported copper concentrations in whole body of *Rana clamitans melanota* and *Rana pipiens* tadpoles as 4-6.9 mg kg^{-1} and lead concentrations were below detection limit. Karasovet al. (2005) also reported that anuran species richness declined with

Figure 7: Bioaccumulation factor of heavy metals in whole body of *P. teraiensis*



increasing concentrations of cadmium, chromium and lead in pond. Birdsall et al. (1986) and Burger & Snodgrass (1998) studied lead concentration in whole body of *Rana catesbeiana* tadpoles and reported that whole body of tadpoles accumulated 20-250 mg kg⁻¹ and 5.43 mg kg⁻¹ of lead concentration respectively. Sparling & Lowe (1996) reported 9.8-15.7 mg kg⁻¹ of copper concentration and 6.7-19.7 mg kg⁻¹ of lead concentration in whole body of *Acris screpitans* tadpoles. In the present study among all the studied tadpoles highest concentrations of copper was found to be 2.07 µg g⁻¹ and lead concentration was found to be 2.42 µg g⁻¹ in whole body of *Microhyla ornata* tadpoles collected from industrial area (Panchgram). It is possible that this species being a filter feeder accumulated more from water and water of the Panchgram site contained higher concentration of lead. Barron (1995) and Singh et al. (2016a) also made similar observations that the tadpoles bioaccumulated these metals more from the water in comparison to sediment. The copper and lead concentrations recorded in the present study were lower than the value given by earlier workers (Kelepertzis et al. 2012). Lead can damage physiological processes and also accumulate in tissue (Sparling et al. 2000). It may lead to malformation of organs, deformities and prolong the development period. Heavy metals in water and sediment was found to affect occurrence of anuran species (Ficken & Byrne, 2013) and negatively correlated to species richness. They also opined that toxic heavy metal contamination is responsible for localised extinction of anuran species in Merri Creek corridor of

Victoria of south east Australia.

As evident from the present study, aquatic bodies of Barak valley are now becoming polluted by heavy metals and other pollutants. Anuran tadpoles may become susceptible to this heavy metal pollution. This is also evident from the study of Kelepertzis et al. (2012) where in it has been suggested that tadpoles be included in monitoring programs as good bioindicators of environmental pollution. From the present study also it can be concluded that the tadpoles can be considered as a good bio-indicators of metals contamination in streams and other aquatic bodies. In different sampling sites there were contamination by pollutants and long time exposure of anuran tadpoles to heavy metals may lead to decline of the anuran population in the region. In view of the importance of anurans in ecosystems, it is recommended that management of aquatic bodies (both natural and man-made) which serve as breeding sites of anurans should be maintained properly to enable their successful breeding. Proper disposal techniques of industrial effluents, agricultural drainage, water containing pesticides, fertilizers and domestic sewage should be practiced to avoid entry of these metals into environment which may lead to severe pollution of water bodies. Creating general awareness regarding the role of anurans in the ecosystems and sensitizing the local people about the importance of conservation of aquatic habitats is needed. Future studies may be taken up on impact of specific heavy metals on different anuran tadpoles to understand acute and chronic effects.

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Duttaphrynus melanostictus.
Photo Credit: Abhijit Das



Table 1: Habitats used by anuran tadpoles from Barak Valley

Sites	Habitat types	Tadpole species
1. Panchgram (IA)	temporary water body	<i>Microhyla ornata</i>
2. Karimganj (RA)	temporary water body	<i>Microhyla ornata</i>
3. Rosekandy (TG)	temporary water body	<i>Microhyla ornata</i>
4. Panchgram (IA)	temporary water body	<i>Hoplobatrachus tigerinus</i>
5. Karimganj (UA)	paddy field	<i>Hoplobatrachus tigerinus</i>
6. Panchgram (IA)	temporary water body	<i>Duttaphrynus melanostictus</i>
7. AUS campus	temporary water body	<i>Duttaphrynus melanostictus</i>
8. AUS campus	temporary water body	<i>Polypedates teraiensis</i>

IA- Industrial area, RA-Rural area, TG- Tea garden, UA-Urban area

Table 2: Heavy metals concentrations (mean±SD) in whole body of tadpoles/water/sediment

Heavy metal concentrations (µg gm ⁻¹ / mg L ⁻¹)*			
Species and sites		Copper (Cu)	Lead (Pb)
<i>Microhyla ornata</i> (MO1) (Panchgram)	Whole body	2.07±0.01	2.42±0.03
	Water	0.025±0.04	0.083±0.02
	Sediment	3.4±0.19	3.46±0.02
<i>Microhyla ornata</i> (MO2) (Karimganj)	Whole body	1.83±0.03	2±0.13
	Water	0.005±0.01	0.017±0.05
	Sediment	0.917±0.04	1.08±0.26
<i>Microhyla ornata</i> (MO3) (Rosekandy)	Whole body	0.6±0.03	0.868±0.03
	Water	0.2±0.08	0.036±0.27
	Sediment	2.43±0.13	2.6±1.54
<i>H. tigerinus</i> (HT1) (Panchgram)	Whole body	1.13±0.01	1.76±0.02
	Water	0.006±0.04	0.008±0.02
	Sediment	3.4±0.19	3.46±0.01
<i>H. tigerinus</i> (HT2) (Karimganj)	Whole body	1.33±0.008	0.83±0.002
	Water	0.004±0.05	0.005±0.04
	Sediment	2.07±0.10	2.96±0.07
<i>D. melanostictus</i> (D1) (Panchgram)	Whole body	0.632±0.005	1.02±0.14
	Water	0.012±0.12	0.04±0.09
	Sediment	4.27±0.06	5.55±0.08
<i>D. melanostictus</i> (DM2) (AUS campus)	Whole body	0.411±0.06	0.024±0.01
	Water	0.011±0.01	0.006±0.03
	Sediment	1.425±0.4	2.21±0.25
<i>P. teraiensis</i> (PT) AUS campus)	Whole body	0.652±0.001	0.654±0.004
	Water	0.003±0.02	0.003±0.03
	Sediment	0.634±0.1	0.893±0.21

Table 3: Significance of differences between species among eight sites as revealed by one-way ANOVA

Species	F-value
<i>Microhyla ornata</i> (Panchgram)	42.49 _{2,14} **
<i>Microhyla ornata</i> (Karimganj)	192.8 _{2,14} **
<i>Microhyla ornata</i> (Rosekandy)	630.7 _{2,14} **
<i>Hoplobatrachus tigerinus</i> (Panchgram)	231.1 _{2,14} **
<i>Hoplobatrachus tigerinus</i> (Karimganj)	253.4 _{2,14} **
<i>Duttaphrynus melanostictus</i> (Panchgram)	234.7 _{2,14} **
<i>Duttaphrynus melanostictus</i> (AUS campus)	550.3 _{2,14} **
<i>Polypedates teraiensis</i> (AUS campus)	11.76 _{2,14} **

**Significant at the 0.01 level

Table 4: Physico-chemical properties of water in different sampling sites.

Site	WT (°C)	AT (°C)	pH	EC (μ s/cm)	DO (mg/L)	FCO2 (mg/L)	TA (mg/L)
1.	25.1±0.6	28.6±0.2	6.8±0.9	95±1.8	1.52±0.2	9.4±0.2	45±2.1
2.	25.6±0.5	28.2±0.7	5.8±0.2	48±1.3	3.5±0.1	8.2±0.3	30±1.3
3.	25.3±0.2	24.8±0.9	6.4±0.6	56±1.2	2±0.3	13.2±0.5	50±1.43
4.	25.3±0.4	28.8±1.6	6.9±0.1	90±0.55	1.55±0.2	9.6±0.7	42.6±2.6
5.	25.6±0.8	28.2±1.3	5.8±0.6	48±1.6	3.5±0.6	8.2±0.4	45±2.6
6.	24.9±0.5	27±0.5	6.2±0.35	85±1.1	1.9±0.4	8.8±0.8	40±2.7
7.	23.5±0.2	28.1±1.1	5.6±0.38	40±0.6	3.7±1.1	9.6±0.2	45±0.8
8.	23.4±1..3	28.4±1.5	5.2±0.2	45±0.59	4.1±1.5	11.6±0.5	40±1.6

Table 5: Significance of differences between physico-chemical parameters (2013-2014) among eight sites as revealed by one-way ANOVA

Parameters	F-value
Water Temperature	63.24 _{7,16} **
Air Temperature	11.15 _{7,16} **
pH	659.2 _{7,16} **
Electrical Conductivity	109.7 _{7,16} **
Dissolved Oxygen	887.04 _{7,16} **
Free Carbon-dioxide	171.05 _{7,16} **
Total Alkalinity	12.25 _{7,16} **

**Significant at the 0.01 level

Table 6: Correlation coefficients between physico-chemical variables and concentration of heavy metals in water from sampling sites

Water parameters	Copper (Cu)	Lead (Pb)
Water temperature	0.165*	0.311*
Air temperature	-0.451	-0.172
pH	0.623*	0.642*
Electrical Conductivity	0.443*	0.622*
Dissolved Oxygen	-0.648*	-0.674*
Free CO2	0.331	0.045
Total alkalinity	0.650	0.357

Dietary Patterns and Niche Overlap in Five Sympatric Anuran Species Assemblages in Uttarakhand region of Western Himalaya

Abstract

The dietary pattern and trophic niche overlap characteristics of anuran assemblage were studied near temporary water pools around foothill regions of Uttarakhand, Western Himalaya. The assemblage consisted of five anuran species viz., three frogs (*Sphaerotheca breviceps*, *Microhyla ornata*, *Fejervarya limnocharis*), and two toads (*Duttaphrynus stomaticus*, *Duttaphrynus melanostictus*). Fieldwork was carried out around foothill region (Dehradun district) of Uttarakhand, Western Himalaya, and located just northern part of India. A total of 146 individuals were sampled. Each frog's stomach was flushed and specimens were released at the capture site during the same night. The diets of different species were analyzed in terms of number, volume (in mm³) and frequency of occurrence of each type of prey. The index of relative importance (IRI) was employed as a measure that reduces bias in the description of animal dietary data. The niche overlaps among these sympatric anuran species were also ascertained. A total of 1427 food items were identified and classified into 28 taxonomic groups. The food items ingested were within the size of 1.9mm-69.06mm. *D. stomaticus* was the most voracious feeder with maximum 14 prey items per sample. The niche overlap among the species is moderate and probably there is no or insignificant competition for food resources between these five species in the places with sympatric distribution. The study revealed feeding habits and diet composition of sympatric species which may help to model the trophic interaction and prey-predator dynamics toward the ecosystem-based amphibian management.

Introduction

Amphibians have attracted the considerable attention of community ecologist for a long time (Inger 1969; Toft 1985). Understanding feeding relationships among amphibian communities are of fundamental interest to herpetologists and ecologists because of the pivot role that amphibians may play in aquatic as well as terrestrial ecosystems (Hirai & Matsui 1999; Luiselli 2006). When in the land, amphibians are thought to be opportunistic predators with their diets just reflecting the availability of food of appropriate size (Duellman & Trueb 1986). Studies have been done in past by different authors on anuran feeding pattern (Daza-Vaca & Castro-Herrera 1999; Botero-Trujillo 2006; Muñoz-Guerrero et al. 2007; Isaacs & Hoyos 2010; Vignoli & Luiselli 2012;

Chowdhary et al. 2016). Investigations of resource utilization by a predator as well as their relationship with their prey and the environment are important for understanding the mechanisms that influence community structure of amphibians. Seasonal abundance of food, size/shape constraints and ecological

Key words:
Amphibian, Diet composition, Niche overlap, Stomach flushing, Sympatric species, Western Himalaya

Forest stream below Roopkund valley, Uttarakhand.
Photo Credit: Abhijit Das

Ashish K Chowdhary, V Bahuguna & S N Bahuguna*

Department of Zoology and Biotechnology, H.N.B. Garhwal University, Srinagar Garhwal- 246 174, Uttarakhand, India

Corresponding author e-mail: profsnbahuguna@rediffmail.com



tolerances are the main factors that influence the dietary pattern of amphibians (Duellman & Trueb 1986). However, the amphibian dietary pattern should be useful for community ecology of amphibian assemblages. For instances, this analysis may be crucial for the benefit of the understanding of assembling rules of living assemblages of species in general.

Anurans have traditionally been considered as suitable model organisms for community ecology studies. Community structures of any ecosystems are altered by the availability of resources and competition in species (Begon et al. 2006). When a species do not have any competitors in the habitat, it can exploit its entire niche width, i.e. its fundamental niche. But if two species compete for the same resources in the same habitat, they will be affected by one another. When competition occurs in the same habitat, both species are restricted to their realized niche. This will lead to interspecific competition resulting decrease survival and fecundity for the competing species (Hardin 1960). The degree of niche differentiation among species in the same trophic level of the ecosystem depends on many factors, been prey availability one of the most relevant (Pianka 1969; Schoener 1989; Duré et al. 2009).

In Western Himalaya, Uttarakhand is an ecologically important area where different habitats comprising hills and valley exist and provide varied kinds of ecological niches. The variety of environmentally important areas supports a wide variety of well-known amphibians. The study of amphibians of this area is significant both from scientific and systematic points of view as well (Ray 1999). Herein, we studied feeding habits of five anuran species viz, three frogs (*Sphaerotheca breviceps*, *Microhyla ornata*, and *Fejervarya limnocharis*), and two toads (*Duttaphrynus stomaticus* and *Duttaphrynus melanostictus*) (Fig.1), specifically addressing the following aims: 1) to describe feeding nature of these anurans, 2) identify and quantify the prey items consumed by them, 3) to calculate the niche width, niche overlap and diversity in the diet of these anurans. All the studied anuran species live sympatrically around



Figure 1a: *Sphaerotheca breviceps*.
Photo Credit: Abhijit Das

the temporary water pools of the study area. Although the study area encompasses diverse anuran fauna (20 different species observed), studies on the dietary patterns of native anuran species are scarce. The present study aims to analyze the competition for food resources among the sympatric species in the places with sympatric distribution.

Materials and Methods

Study Area: Fieldwork was carried out around foothill area i.e. Dehradun district of Uttarakhand, Western Himalaya (Fig. 1). Dehradun comprises montane, submontane and valley covering an area of about 2000 sq. km. situated between latitude 29°58' and 31°02' N, longitude 77°35' and 78°20' E. The climate is partly tropical and partly temperate. During monsoon it rains from mid-June to mid-August, mean annual temperature varies from 0°C to 40°C. The total average rainfall is about 2000mm per annum, 85 % of which is recorded in the months of June, July, August and September (Ray 1999).

Sample collection: A total of 146 individuals were analyzed: 29 *S. breviceps*, 21 *M. ornata*, 26 *F. limnocharis*, 31 *D. stomaticus*, and 39 *D. melanostictus*. The field work was carried out for two breeding seasons i.e. from June to September (2015-2016) at evening hours (2000 hrs to 2200 hrs). Nocturnal Visual Encounter Method was employed for sampling (Heyer et al. 2014). A visual survey was also carried out to study feeding habitat and feeding nature of studied specimen (Bahuguna et al. 2014;

Chowdhary et al. 2014).

Sampled frog's stomach was flushed and specimens were released at the capture site during the same night (Solé et al. 2005). As soon as the stomach contents were collected, the individuals were released in the provenience biotope. Stomach contents were transferred to vials, fixed in 70% ethanol and later analyzed under a stereomicroscope (Olympus SZX12; Range of magnification 9-55X). Food items were identified to the lowest taxonomic level (Roy & Brown 2004; Gibb & Oseto 2006), and then photographed with a digital camera (Nikon D5500). Prey items were classified following order level, with the exception of Hymenoptera, which was classified as Formicidae and Non-Formicidae. Prey remains that could not be identified were grouped in the category "Unidentified".

Data Analysis and analytical

procedure: The diets of different anurans were analyzed in terms of number, volume (in mm³) and frequency of occurrence of

each type of prey. Prey items were numbered, and each prey's width (in mm) and length (in mm) were evaluated with digital Vernier caliper (Aerospace) to the nearest 0.1 mm accuracy. Completely preserved items were measured and had their volume V (in mm³) calculated using the formula for ellipsoid bodies (Griffiths & Mylotte 1987): $V = \frac{4}{3} \pi (L/2) (W/2)^2$

Where: L = prey length and W = prey width.

We obtained the frequency of occurrence of each prey category in the diet dividing the number of stomachs which contained that category by the total number of stomachs analyzed, except the empty ones. The index of relative importance (IRI) was employed as a measure that reduces bias in the description of animal dietary data (Pinkas et al. 1971);

$$IRI = (N\% + V\%)F\%$$

Where $N\%$ = Numerical percentage, $V\%$ = Volumetric percentage, $F\%$ = Frequency of occurrence percentage and IRI = Index of relative importance.

Figure 1b: *Microhyla cf. ornata* from elevation 1300 m
Photo Credit: Naitik Patel



In order to compare the trophic niche breadth, the standardized Shannon-Weaver entropy index J' was used (Shannon & Weaver 1949):

$$J' = H' / \ln(n) \text{ whereby}$$

$$H' = - \sum p_i \ln(p_i)$$

p_i is the relative abundance of each prey category, calculated as the proportion of prey items of a given category to the total number of prey items (n) in all compared studies. To make H' index number more biological sense, it was converted into the Effective Number of Species (ENS), which is the real biodiversity and allows to compare the biodiversity with other communities. A community with Shannon index of H' has an equivalent diversity as a community containing equally-common species of $\exp(H')$, the ENS .

The niche breadth was obtained through Levins' standardized index (Krebs 1999), in which the value of Levins' measure (B) was first obtained. Levins' measure was calculated by the following equation:

$$B = 1 / \sum p_i^2$$

where, B is Levins' niche breadth measure and P_i is the fraction of item i in the diet.

Levins' measure was then standardized on a scale of 0-1.0 by the following equation:

$$B_A = (B-1) / (n-1)$$

where, B_A corresponds to Levins' standardized niche breadth, B is Levins' niche breadth measure, and n is the number of possible resource states. Levins' standardized niche breadth ranges from 0 (the narrowest amplitude), when there is exclusive use of a single resource category, to 1 (the broadest amplitude), when all categories are equally used; the species is considered to have a wide niche breadth when $BA \geq 0.5$ (Krebs 1999; Caldart et al 2012).

The simplified Morisita index proposed by Horn(1966) is another similarity index that we used to measure niche overlap, also called as Morisita-Horn index. It is calculated from the formula:

$$C_H = (2 \sum_i^n p_{ij} p_{ik}) / (\sum_i^n p_{ij}^2 + \sum_i^n p_{ik}^2)$$

where, CH = Simplified Morisita Index of overlap between species j and species k , p_{ij} = Proportion resource i is of the total resources used by species j , p_{ik} = Proportion resource i is of the total resources used by species k , and n = Total number of resource states ($i = 1, 2, 3, \dots, n$)

Figure 1c: *F. teraiensis*
Photo Credit: Abhijit Das



Results

Descriptive analysis of the diets

A total of 146 individuals were analyzed: 29 *S. breviceps*, 21 *M. ornata*, 26 *F. limnocharis*, 31 *D. stomaticus*, and 39 *D. melanostictus* (Fig.2). The index of vacuity (individuals with empty stomach*100/ total number of individuals) was nearly 14 % (n = 4) in *S. breviceps*, 24 % (n = 5) in *M. ornata*, 19 % (n = 5) in *F. limnocharis*, 23 % in *D. stomaticus* (n = 7), and 28 % in *D. melanostictus* (n = 11). A total of 1427 food items was identified and classified into 28 taxonomic groups. The food items ingested were within the size of 1.9mm-69.06mm. *D. stomaticus* were the most voracious feeder with maximum 14 prey items per sample (Table 1). The larvae and adults were separated for Coleoptera, Lepidoptera, and Diptera, because it was considered the fact that, they represent different categories, as far as the mobility and the provenience environment are concerned.

The 276 consumed animals by *S. breviceps* were grouped in 20 categories of invertebrates. Diptera was the most important prey item in terms of number (13.77%), followed by Formicidae (11.23%), Diptera larvae (10.51%), Scolopendromorpha (9.42%) and Spirobolida (7.61%). Volumetrically, the greatest contribution was from Lepidoptera (31.02%), followed by Orthoptera (21.12%), Lepidoptera larvae (12.80%), Trichoptera (8.88%) and Coleoptera (6.12%). The most frequent items were Diptera (15.36%), Diptera larvae (12.43%), and Orthoptera (10.76%). Orthoptera (297.41), Diptera (219.49), Diptera larvae (140.71), Scolopendromorpha (92.97), Coleoptera (88.17), and Spirobolida (70.91) were the items with the highest index of relative importance (Table 2a, Fig.3). The Shannon-Wiener measure of niche breadth, H' was observed 2.70 (ENS= 15) and Evenness measure of the Shannon-Wiener function, J' was 0.903 (Table 3).

M. ornata consumed total 167 invertebrate individuals which represented only six prey categories. Formicidae was the most important prey item in terms of number (61.08%), followed by Isoptera (24.55%) and Coleoptera (7.19%). Volumetrically, the greatest contribution was from Coleoptera (31.40%), followed by Hemiptera (25.11%),



Figure 1 d: Egg laying site of *Duttapahyrus melanostictus*.
Photo Credit: Abhijit Das

Isoptera (19.23%), Diptera (11.18%), and Formicidae (9.20%). The most frequent items were Formicidae (63.18%), Isoptera (19.05%), and Coleoptera (5.26%). Formicidae (4440.29), Isoptera (834.01), Coleoptera (202.98) and Hemiptera (118.53) were the items with the highest index of relative importance (Table 2a, Fig.3). The Shannon-Wiener measure of niche breadth, H' was observed 1.09 (ENS= 3) and Evenness measure of the Shannon-Wiener function, J' was 0.609 (Table 3).

Furthermore, 232 consumed animals by *F. limnocharis* were grouped in 17 categories of invertebrates. Formicidae was the most important prey item in terms of number (24.12%), followed by Orthoptera (16.45%) and Isopoda (11.54%). Volumetrically, the greatest contribution was from Orthoptera (31.67%), followed by Lepidoptera (16.21%), Haplotaxida (10.45%) and Lepidoptera larvae (9.67%). The most frequent items were Orthoptera (25.16%), Isopoda (15.27%), and Coleoptera (11.67%). Orthoptera (1210.70), Isopoda (263.10), Scolopendromorpha (93.39), Lepidoptera larvae (90.86), and Formicidae (78.34) were the items with the highest index of relative importance (Table 2a, Fig.3). The Shannon-Wiener measure of niche breadth, H' was observed 2.45 (ENS= 12) and Evenness measure of the Shannon-Wiener function, J' was 0.863 (Table 3).

From 31 *D. stomaticus* stomachs, we identified 430 food items that represented 18 prey categories. The most common prey was Formicidae, which had the highest frequency of occurrence of 14.95% and followed by Araneae (12.49), and Coleoptera



Figure 1e: *Duttaphrynus melanostictus*.
Photo Credit: Abhijit Das

(11.65%). Items consumed in terms of the number were: Formicidae (43.16%), Coleoptera-adults (8.39%), and Araneae (7.22%). Lepidoptera (28.55%), followed by Orthoptera (27.12%), and Lepidoptera larvae (11.82%) had the greatest contribution volumetrically. Similarly, according to the IRI, Formicidae were the most important items at a value of 673.35, Orthoptera (176.33) were the second most important item, followed by Coleoptera (158.67), Araneae (98.92), and Lepidoptera (95.10) (Table 2b, Fig.3). The Shannon-Wiener measure of niche breadth, H' was observed 2.14 (ENS= 9) and Evenness measure of the Shannon-Wiener function, J' was 0.742 (Table 3).

D. melanostictus (N=39) individuals consumed total 322 food items which were grouped into 25 food item categories. Formicidae was the most important prey item in terms of number (18.95%), followed by Coleoptera (14.29%) and Coleoptera larvae (9.62%). Volumetrically, the greatest contribution was from Orthoptera (19.36%), followed by Odonata (17.29%) and Lepidoptera (16.30%). The most frequent items were Coleoptera (15.97%), Coleoptera larvae (11.81%), and Formicidae (11.58%). Coleoptera (269.89), Formicidae (233.22), Orthoptera (158.63), Coleoptera (158.61), and Lepidoptera (119.93) were the items with the highest index of relative

importance (Table 2b, Fig.3). The Shannon-Wiener measure of niche breadth, H' was observed 2.77 (ENS= 16) and Evenness measure of the Shannon-Wiener function, J' was 0.86 (Table 3).

Food niche breadth and overlap

S. breviceps presented the largest niche breadth (BA = 0.617), followed by *F. limnocharis* (BA = 0.465) and *D. melanostictus* (BA = 0.439). The lowest niche breadth was presented by *D. stomaticus* (BA = 0.216) followed by *M. ornata* (BA = 0.253). Among the five sympatric anurans, *D. stomaticus* presented the highest food overlap indices with *M. ornata* (CH= 0.881), *D. melanostictus* (CH= 0.763), and *F. limnocharis* (CH= 0.756), followed by *D. melanostictus* with *F. limnocharis* (CH= 0.745) and *S. breviceps* with *D. melanostictus* (CH= 0.680), and *F. limnocharis* (CH= 0.626) (Table 4).

Discussion

Anurans can be considered opportunistic predators when the diet has some relationship with prey availability in the environment (Duellman and Trueb, 1994). Arthropods such as Formicidae, Coleoptera, Chilopoda, Isopoda, and Araneae are recognized as being an important diet of medium-sized frogs (Van Sluys et al. 2001; Dietl et al. 2009). We hypothesized that the five sympatric anuran species studied,

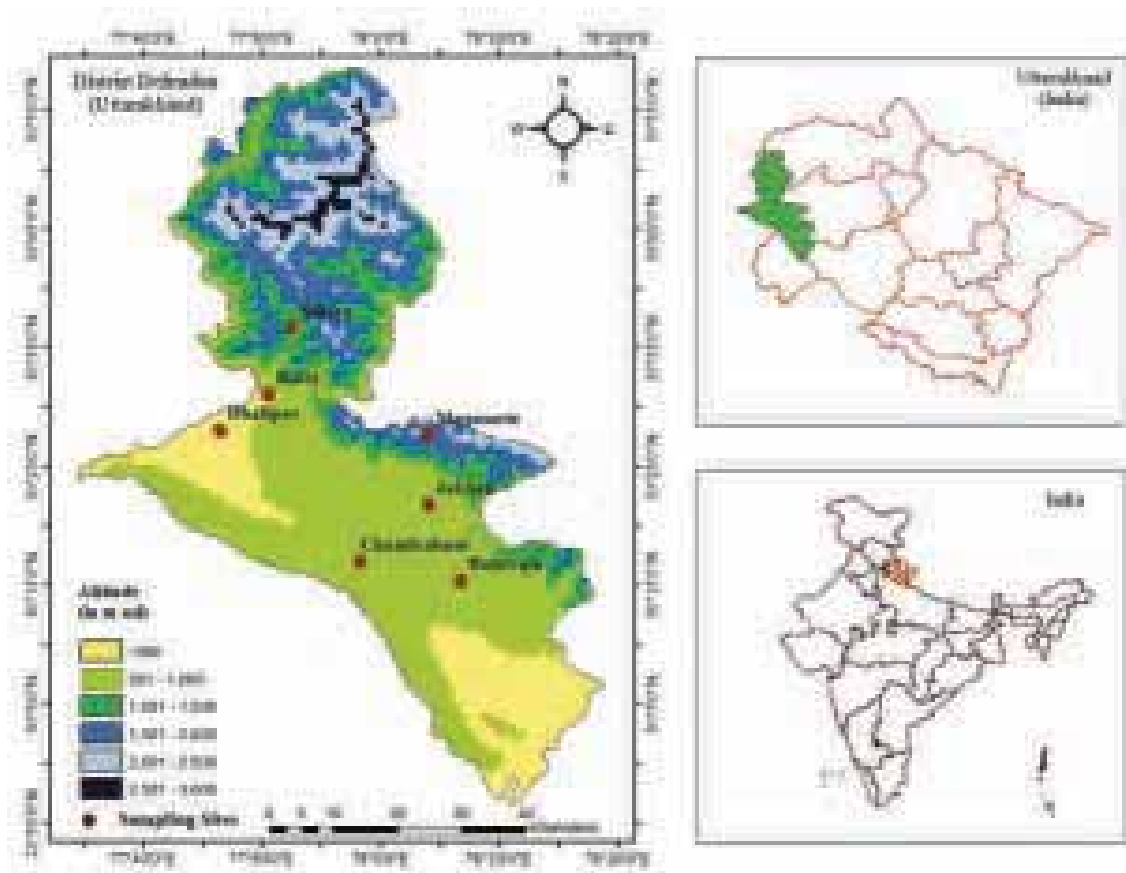


Figure 2: Location map showing different sampling sites in Dehradun region of Uttarakhand, Western Himalaya

exploit the same habitats and consume the same types of preys because they were observed feeding together in the same habitats.

The five sympatric anuran species *S. breviceps*, *M. ornata*, *F. limnocharis*, *D. stomaticus*, and *D. melanostictus* are morphologically different species, but they temporally occur in sympatry before hibernation. Owing to different body sizes, their diets were different. Among frogs, *S. breviceps* consumed Orthoptera, Diptera, Diptera larvae, Scolopendromorpha, Coleoptera, and Spirobolida in abundance. *F. limnocharis* preferred mostly Orthoptera and Isopoda, while *M. ornata* feeding was restricted mostly on Formicidae and Isoptera as major food constituents. Among toad species, both *D. stomaticus* and *D. melanostictus* preferred Formicidae, Orthoptera, and Coleoptera as important food items. Plant seeds were observed only in the diet of toads.

The five sympatric anuran species demonstrated a varied food spectrum, especially *D. melanostictus* and *S. breviceps*

with maximum twenty-five and twenty prey taxa respectively, while *M. ornata*, with only six prey taxa consumed (Fig.3). Considering the type and proportion of prey in the diet, Indian burrowing frog *S. breviceps* and common Indian toad *D. melanostictus* could be considered generalists with a foraging strategy that could be classified as intermediate along the extremes of sit-and-wait and actively foraging. The five studied anuran species also selected adult Coleoptera, and for that, the predator would change from sit-and-wait behavior to actively foraging. The typical sit-and-wait predator has a low metabolic rate, the encounter rate with the active prey is low, niche breadth is wide, and the sensory mode is visual to locate prey items (Perry & Pianka 1997; Yu & Guo 2012).

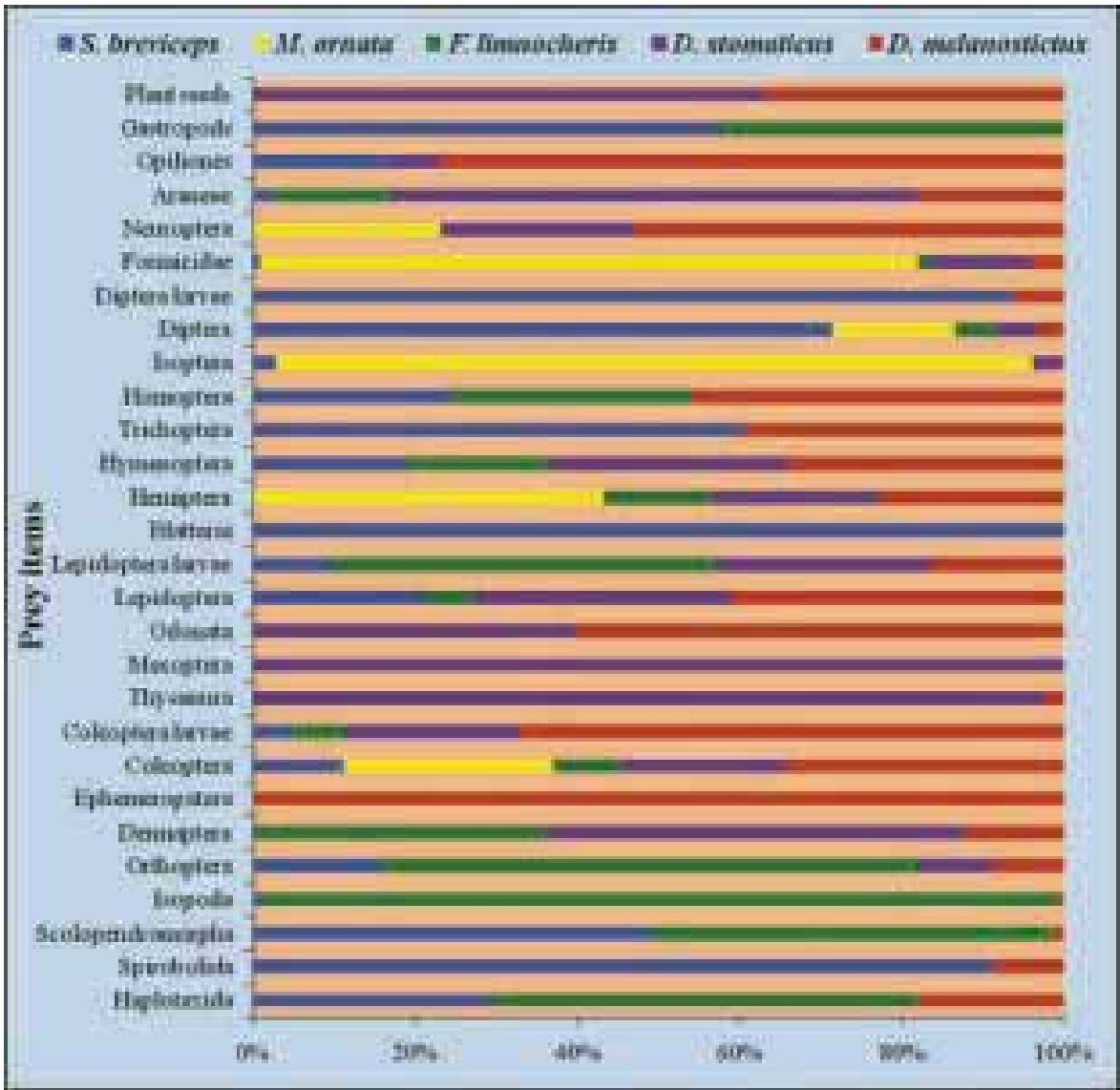
Anuran skin secretions are a rich source of biologically active compounds including a wide variety of chemical substances such as terpenes, amines, alkaloids, steroids, peptides and proteins (Mebs et al. 2010). Ants are generally considered to be of poor nutritional value (Redford & Dorea 1984; Withers & Dickman 1995; Meyers & Herrel

2005). Many ants, beetles and some other arthropods, such as millipedes also produce noxious chemicals that make them unpalatable to many potential predators (Skaife et al. 1979). An interesting finding of this study is the relatively high incidences of predation on ants, beetles, and millipedes by studied anuran species. Some anurans like *S. breviceps*, *D. stomaticus* and *D. melanostictus* actually incorporate the noxious chemicals produced by such arthropods into their own defensive mechanisms, and thus selectively prey on such invertebrates (Daly et al. 2007). The predation on ants, millipedes, and beetles by anurans may play a role in their defensive mechanisms and should also be explored.

The results of present study showed that the

trophic niche of *D. stomaticus* presented the highest food overlap indices with *M. ornata*, *D. melanostictus*, and *F. limnocharis*, followed by *D. melanostictus* with *F. limnocheris* and *S. breviceps* with *D. melanostictus*, and *F. limnocheris*, which indicated a similarity between the diets. The three studied frog species use different microhabitats than the toads, so we suggest that competitive interactions between these sympatric species are infrequent. For toads and the three frog species, trophic niche overlap was low, and the differences in prey volume would favor the coexistence of five temporary congregated species in an effective way same as observed and discussed earlier in *Bufo gargarizans*, *Rana guentheri*, and *Rana limnocharis* by Yu & Guo (2012).

Figure 3: Proportion of prey items consumed based on index of relative importance (IRI) by five studied sympatric anuran species, viz, three frogs (*S. breviceps* Sb, *M. ornata* Mo, *F. limnocharis* Fl), and two toads (*D. stomaticus* Ds, *D. melanostictus* Dm).



In conclusion, we could say that these studied five sympatric species have common feeding behavior, but there are certain differences in their trophic niche. The niche overlap is moderate and probably there is no or insignificant competition for food resources among these five species in the places where they found in sympatry. The study revealed feeding habits and diet composition of sympatric anuran species which may help to model the trophic interaction and prey-predator dynamics toward the ecosystem-based amphibian management. The data obtained on the feeding behavior and preference of the prey species would certainly help in executing ecological management program with a view to restoring the biodiversity of the Himalayan region.

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Table 1: The total number of prey, the average and maximum number of prey items/ samples; the amount of terrestrial preys for the whole period in stomach contents of five sympatric anuran species, three frogs (*S. breviceps* Sb, *M. ornata* Mo, *F. limnocharis* Fl), and two toads (*D. stomaticus* Ds, *D. melanostictus* Dm) in Dehradun region of Uttarakhand, Western Himalaya.

	Sb	Mo	Fl	Ds	Dm
Total Sample Size	29	21	26	31	39
Individuals with empty stomach	4	5	5	7	11
Total prey taxa present	20	06	17	18	25
Total no. of preys	276	167	232	430	322
Average no. of prey items / samples	9	8	9	14	8
Terrestrial preys (%)	89.49	100	100	100	95.66
Aquatic preys (%)	10.51	-	-	-	4.34
Maximum length of prey item (mm)	56.3	31.45	43.01	62.23	69.06
Minimum length of prey item (mm)	3.5	1.9	2.6	3.7	3.9
Plant seeds (%)	-	-	-	2.53	2.50

Table 2a: Prey categories with their respective absolute values and relative abundance (%N), frequency of occurrence (%O), volume (%V) and index of relative importance (IRI) of each prey item in the diet of three sympatric frogs *S. breviceps*, *M. ornata* and *F. limnocharis* in foothill region i.e. Dehradun district of Uttarakhand, Western Himalaya.

	Sb (29)				Mo (21)				Fl (26)			
Order	N %	V %	F %	IRI	N %	V %	F %	IRI	N %	V %	F %	IRI
Clitellata												
Haplotaxida	2.9	3.74	4.12	27.36					3.49	10.45	3.45	48.09
Diplopoda												
Spirobolida	7.61	1.08	8.16	70.91								
Chilopoda												
Scolopendromorpha	9.42	0.46	9.41	92.97					6.83	8.38	6.14	93.39
Malacostraca												
Isopoda	0.72	1.34	1.27	2.62					11.54	5.69	15.27	263.10
Insecta												
Orthoptera	6.52	21.12	10.76	297.41					16.45	31.67	25.16	1210.70
Dermaptera									3.89	0.39	2.30	9.84
Ephemeroptera												
Coleoptera	6.16	6.12	7.18	88.17	7.19	31.40	5.26	202.98	4.09	1.60	11.67	66.40
Coleoptera larvae	2.9	1.18	2.78	11.34					2.75	1.13	3.78	14.67
Thysanura												
Mecoptera												
Odonata												
Lepidoptera	1.81	31.02	1.93	63.36					0.42	16.21	0.93	15.47
Lepidoptera larvae	1.09	12.80	1.35	18.75					5.25	9.67	6.09	90.86
Blattaria	4.35	5.65	4.08	40.80								
Hemiptera					3.59	25.11	4.13	118.53	3.67	3.14	5.26	35.82
Hymenoptera*	4.71	0.28	2.78	13.87					2.83	0.37	3.77	12.06
Trichoptera	0.36	8.88	1.08	9.98								
Homoptera	3.99	0.07	1.46	5.93					2.53	0.95	2.11	7.34

Order	Sb (29)				Mo (21)				Fl (26)			
	N %	V %	F %	IRI	N %	V %	F %	IRI	N %	V %	F %	IRI
Isoptera	6.52	0.19	3.68	24.69	24.55	19.23	19.05	834.01	4.93	0.21	0.27	1.39
Diptera	13.77	0.52	15.36	219.49	2.99	11.18	3.26	46.19	3.67	0.64	3.80	16.38
Diptera larvae	10.51	0.81	12.43	140.71								
Formicidae	11.23	0.66	4.18	49.70	61.08	9.20	63.18	4440.29	24.12	0.83	3.14	78.34
Neuroptera					0.60	0.29	0.56	0.50				
Arachnida												
Araneae	2.9	0.59	1.11	3.87					2.56	4.37	3.17	21.97
Opiliones	1.81	0.07	1.67	3.14								
Gastropods	0.72	0.64	2.08	2.83					0.98	0.57	1.33	2.06
Plant seeds												
Unidentified		2.78	3.13			3.59	4.56			3.73	2.36	

*Non- Formicidae

Table 2b: Prey categories with their respective absolute values and relative abundance (%N), frequency of occurrence (%O), volume (%V) and index of relative importance (IRI) of each prey item in the diet of two sympatric toads *D. stomaticus* and *D. melanostictus* in foothill region i.e. Dehradun district of Uttarakhand, Western Himalaya.

Order	Ds (31)				Dm (39)			
	N %	V %	F %	IRI	N %	V %	F %	IRI
Clitellata								
Haplotaxida					2.79	3.22	2.78	16.71
Diplopoda								
Spirobolida					1.55	1.68	2.08	6.72
Chilopoda								
Scolopendromorpha				0.31	3.45	0.69	2.59	
Malacostraca								
Isopoda					0.93	0.46	1.39	1.93
Insecta								
Orthoptera	3.60	27.12	5.74	176.33	5.90	19.36	6.28	158.63
Dermaptera	2.53	0.35	4.90	14.11	4.03	0.80	0.69	3.33
Ephemeroptera				0.63	0.36	0.69	0.68	
Coleoptera	8.39	5.23	11.65	158.67	14.29	2.61	15.97	269.89
Coleoptera larvae	5.66	1.25	7.58	52.38	9.62	3.81	11.81	158.61
Thysanura	3.71	0.82	5.52	25.01	0.31	0.54	0.69	0.59
Mecoptera	0.19	1.05	0.61	0.76				
Odonata	0.58	5.94	1.22	7.95	0.31	17.29	0.69	12.14
Lepidoptera	2.53	28.55	3.06	95.10	5.27	16.30	5.56	119.93
Lepidoptera larvae	2.14	11.82	3.68	51.37	2.80	6.19	3.49	31.38
Blattaria								
Hemiptera	4.10	2.96	7.97	56.27	4.04	6.01	6.25	62.81
Hymenoptera*	3.32	1.02	4.90	21.27	3.72	0.54	5.68	24.20
Trichoptera					0.93	3.75	1.39	6.51
Homoptera					2.80	2.61	2.08	11.25
Isoptera	6.44	0.26	4.29	28.74	2.80	0.15	1.39	4.10
Diptera	2.73	0.21	4.90	14.41	3.41	0.40	2.78	10.59

Table 2b:

Prey categories with their respective absolute values and relative abundance (%N), frequency of occurrence (%O), volume (%V) and index of relative importance (IRI) of each prey item in the diet of two sympatric toads *D. stomaticus* and *D. melanostictus* in foothill region i.e. Dehradun district of Uttarakhand, Western Himalaya.

Order	Ds (31)				Dm (39)			
	N %	V %	F %	IRI	N %	V %	F %	IRI
Diptera larvae				4.34	0.09	2.08	9.21	
Formicidae	43.16	1.88	14.95	673.35	18.95	1.19	11.58	233.22
Neuroptera	0.39	0.47	0.61	0.52	0.62	0.20	1.39	1.14
Arachnida								
Araneae	7.22	0.70	12.49	98.92	4.98	0.83	4.86	28.24
Opiliones	0.78	0.13	1.22	1.11	2.17	1.91	3.48	14.20
Gastropods								
Plant seeds	2.53	0.83	2.45	8.23	2.50	0.93	1.39	4.77
Unidentified		9.41	2.26			5.32	2.84	

*Non- Formicidae

Table 3:

The Shannon-Wiener measure of niche breadth (H'); Evenness measure of the Shannon-Wiener function (J'); Levin's Measure of niche breadth (B); and standardized Levin's Measure of niche breadth (BA) of prey items in the diet of five sympatric anuran species, three frogs (*S.breviceps*, *M. ornata*, *F. limnocharis*), and two toads (*D. stomaticus*, *D. melanostictus*) in Dehradun region of Uttarakhand, Western Himalaya.

	Shannon-Wiener Function		Levin's Measure	
	H'(*)	J'	B	BA
<i>S.breviceps</i>	2.70 (15)	0.903	12.723	0.617
<i>M. ornata</i>	1.09 (3)	0.609	2.266	0.253
<i>F. limnocheris</i>	2.45 (12)	0.863	8.447	0.465
<i>D. stomaticus</i>	2.14 (9)	0.742	4.677	0.216
<i>D. melanostictus</i>	2.77 (16)	0.860	11.559	0.439

* Effective Number of Species (ENS)

Table 4:

Morisita- Horn's food niche overlap (CH) among five sympatric anuran species, three frogs (*S. breviceps*, *M. ornata*, *F. limnocharis*), and two toads (*D. stomaticus*, *D. melanostictus*) in Dehradun region of Uttarakhand, Western Himalaya.

	<i>S. breviceps</i>	<i>M. ornata</i>	<i>F. limnocharis</i>	<i>D. stomaticus</i>
<i>M. ornata</i>	0.358			
<i>F. limnocheris</i>	0.626	0.588		
<i>D. stomaticus</i>	0.480	0.881	0.756	
<i>D. melanostictus</i>	0.680	0.513	0.745	0.763

Ex situ Conservation and Management of Amphibians

Abstract

Amphibians are facing large-scale population decline and extinction globally. In this context, ex-situ conservation became a popular practice. In this article, management intervention for ex situ propagation of Indian amphibians are discussed. Biotic and abiotic factors responsible for breeding success are listed. Captive breeding measures are delineated 16 anuran species and one urodela are prioritized for captive breeding programme. 23 Zoological parks across India are identified for ex situ management of amphibians.

Introduction

Worldwide, amphibian populations are declining (Alford, 1999), and India has about 417 species and more than 70% of them are endemic to the region. It also harbours more threatened species of amphibian than any other country in the Indo Malayan realm (Stuart et al. 2005). The Western Ghats and Eastern Himalaya are recognised biodiversity hotspots, with high levels of amphibian endemism (Myers et al. 200 & Gunawardene et al. 2007). The amphibians of the Western Ghats are enigmatic and new species are being described with astonishing frequency, it is estimated that just 50% of amphibians in the South Asian region have been described (Molur, 2008) with new amphibian genera being described as recently as 2013 (Abraham et al. 2013).

Indian Zoos could play a pivotal role in the conservation management of the countries threatened amphibians. In addition zoos are ideally placed to educate the visiting the public about amphibians and the threats that they face. Currently amphibians are underrepresented in Indian Zoos and only one species i.e. *Tylototriton himalayanus* is only maintained by one institution, Padmaja Naidu Himalayan Zoological Park in Darjeeling. The Central Zoo Authority

recognises the need to increase capacity to house amphibian in zoos.

In past there have been efforts documented for the breeding of amphibians, particularly of Verrucose frog, Large wrinkled frog and Ranids at Coimbatore, India (Gupta, 1998). Subsequently a hand book was developed as part of Project on Captive Management and Husbandry of selected Amphibians of Western Ghats funded by Gerald Durrell Wildlife Conservation Trust, UK during 1997-1998 (Gupta, 1998).

Over the years CZA has paved way for prioritisation of species and preparation of a plan for coordinated conservation breeding for Indian amphibians. With ecology and biology of many amphibians in India

Key words:

Amphibians, ex situ, Conservation, Housing, Upkeep, Captive Management, Amphibia, threatened, captive breeding, management, conservation.

Rhacophorus pseudomalabaricus, a charismatic yet threatened amphibian species.
Photo Credit: Sandeep Das

Brij Kishor Gupta

Evaluation and Monitoring Officer
Central Zoo Authority
Ministry of Environment. Forests and
Climate Change, Government of India
B-1 Wing, 6th Floor, Pt.
Deen Dayal Antodaya Bhawan
CGO Complex, New Delhi 110 003

Corresponding author e-mail: brijkishor68@yahoo.com





remains unknown, it is potentially difficult to keep, establish and breed Indian amphibians. We strongly urge young biologists and zoo professionals to gather information through targeted studies on species in the field before embarking on captive breeding programmes. A step in that direction would be to identify a model amphibian species for the zoo to practice husbandry techniques with, nevertheless a vital first step for promoting amphibian conservation breeding in Indian zoos.

The Central Zoo Authority has made several attempts to strengthen conservation breeding programme for amphibians, by providing training to zoo professionals and promoting ex-situ conservation efforts in different Indian Zoos. In February 2008, in the International Conference organized by CZA on India's Conservation Initiative where in the conservation breeding programme of Himalayan Salamander was reviewed. In January 2009, workshop on ex-situ conservation for amphibians was organized by CZA in Mysore where preliminary attempts were made to prioritize amphibians for conservation breeding in Indian zoos. In December 2013, the CZA organised a workshop "Building National Capacity for ex-situ Amphibians

Management and Conservation" in Guwahati, where a list target and practice species of amphibians were identified. During this workshop the Central Zoo Authority with the assistance of the Durrell Wildlife Conservation Trust and the Zoological Society of London has strengthened national capacity in amphibian management. More than 80 delegates from all over India representing nearly 40 institutions participated in these workshops. The participants were exposed to the specific requirements of amphibians in the design and management of ex-situ facilities. Participants developed hands-on skills in enclosure design, the management of water flow and quality, temperature and light within the captive facility and the principles of amphibian nutrition, reproduction, bio-security and conservation education. The potential contribution that ex situ amphibian management could provide to in situ amphibian conservation was also covered. As an outcome of this workshop a publication titled as "Ex situ Management of Amphibians" was published during 2015 (Gupta et al. 2015). As many species of amphibians are declining rapidly, it is high time to initiate the efforts for the ex situ management of amphibians.

Figure 1: Glass fronted terrarium for amphibians with live plants.



Figure 2: Himalayan salamander exhibit
Managing Amphibians Ex situ

Today modern zoological facilities strive to provide for the complex needs of animals and aim, as far as possible, to reproduce the natural environment and habitat which should meet the natural needs of the species in their care. However, those who care for wild animals in zoos understand that for many animals, life in a zoo, no

matter how well managed, involves compromise. This does not mean that the welfare and care of animals in zoos are necessarily compromised, and does not minimize the ongoing efforts to meet the needs and domains of animal welfare for all species.



Figure 3: Wooden logs and live vegetation used in terrarium for housing amphibians.



Figure 4: enriched terrarium type exhibit for frogs. Terracotta tiles are being provided for hiding frogs and this keeps surface moist too.

The high quality of care provided in a captive environment significantly reduces the time an animal must spend in these pursuits, but does not address the behavioural needs associated with these activities. It has become necessary to provide alternative methods of stimulating natural behaviour of the species housed therein to meet both the mental and physical needs of the captive animal.

Enrichment has become essential in zoos to promote species-typical behaviour by providing animals with a naturalistic environment as of similar to wild situations. That environment may include activities that are both challenging and time consuming and may serve several functions, for achieving so, we should know following:

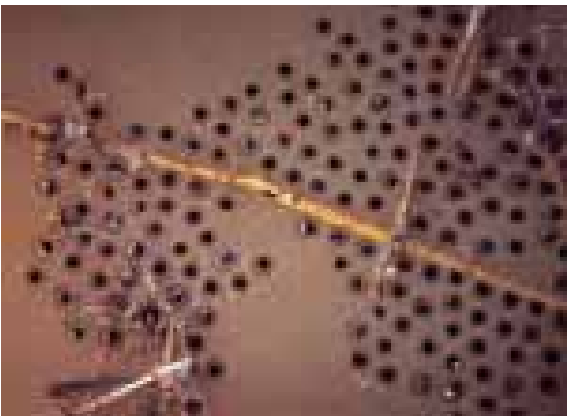
Know your species

Amphibians have complex and varied husbandry requirements and are not always easy to maintain and breed. In captivity keepers should aim to replicate wild micro-environments and conditions as closely as possible and where feasible captive management should be informed by field

data as well as knowledge of the biology of the species. Replicating wild conditions may improve captive breeding success. Unfortunately, as little is currently known about the micro-climates utilised by most amphibian species in the wild keepers may have to collect these data for themselves or correspond with field biologists in order to obtain these very important data.



An ideal amphibian exhibit



Eggs with jelly reared in captivity

Figure 6: Spade footed burrowing frog *Sphaerotheca breviceps* another target species for conservation breeding initiatives.



Enclosure

Enclosure dimensions will depend on the type of species being kept and typically larger and more active amphibians require larger sized enclosures. Arboreal species should be provided with tall enclosures (E.g. *Polypedates* spp. *Rhacophorus* spp. *Pedostibes tuberculosus*) and terrestrial species (e.g. *Duttaphrynus* spp. *Fejervarya* spp. *Clinotarsus curtipes*) should be provided with a greater amount floor space; enclosure height is less important for these. Note that some species can be incredibly active and jumpy and when they are maintained in small enclosures they may be prone to rostral abrasions.

There are several important factors to consider when selecting an enclosure for amphibians. Enclosures must be escape proof. Due to their permeable skin amphibians may quickly desiccate and die if they escape. The construction material should be easy to clean and preferably be smooth. Wood is not appropriate as it may warp with high humidity. It is not possible to disinfect wood during routine cleaning so this material should be avoided. Many chemicals used to seal wood are toxic to

amphibians. Plastic enclosures are a suitable alternative. Any plastic should be food grade as many plastics can leach substances that may be detrimental to amphibian health over time. Plastics may also become brittle when exposed to UVB radiation (an important component of amphibian husbandry). Plastic can be easily cleaned and disinfected. Glass is another alternative; it is readily available, easy to clean and disinfect although glass may break easily. Construction of enclosures using brick and cement should be avoided as these materials may leach minerals into water bodies and may be detrimental to water quality. Amphibians are ectothermic and require a gradient of UVB radiation, temperature; visible light and humidity, therefore amphibian enclosures will need to be lit and appropriate sized meshed apertures need to provide air exchange in the roof or lid of the enclosure. Keeper access is also an important factor to consider for ease of maintenance while minimizing chances for escapes while servicing the enclosure. Ideally, drainage holes with taps will need to be provided to facilitate easy cleaning.

Figure 5: Indian balloon frog *Uperodon globulosum* is a target species for conservation breeding initiatives



Substrate

There are a huge variety of substrates available for amphibians and the type of substrate used will depend on the species being maintained. It is important to consider the depth of the substrate. Fossorial amphibians (e.g. caecilians, *Sphaerotheca* spp.) may require deep substrates for them to burry into. The humidity of the substrate and its potential of water absorption and retention also needs to be considered. As amphibians have permeable skin (and drink through their skin) there should be a moist area of substrate at all times.

A gradient of humidity in the enclosure should be provided which will allow the amphibian to self-regulate its water balance. For the majority of species water logged substrates are not optimal. Amphibians do need to be given the opportunity to sit in dryer areas within the enclosure and failing to provide some dry areas could result in serious health issues. Substrates should be changed as and when needed. Some amphibians may ingest substrate; this is not always a problem unless the amphibian ingests something it is unable to pass through its gastrointestinal tract (e.g. pebbles / strings of moss), the feeding behaviour of the amphibian should therefore be considered when selecting a substrate. Some substrates can be very powdery and may stick to the skin of an amphibian and interfere with the function of the skin (gaseous exchange etc.). Substrates for surface active amphibians should therefore be compacted to minimize this.

Refugia / furnishings

All amphibians should be provided with refugia. The provision of multiple refugia within an enclosure will make the enclosure more heterogeneous. This is of fundamental importance as amphibians will select sites to regulate their temperature and water balance. Cover objects and visual barriers are also important, amphibians are eaten by a wide variety of other organisms and keeping them in very open enclosures with limited cover can result in severe rostral abrasions, stress and disease. For hides to have the desired effect of providing

adequate humidity and to reduce stress, they will need to be individually selected to fit the target species' size and shape so that the animals fit in or under snugly. Arboreal species will require branches as perch / rest sites and some species will benefit from live plants (e.g. *Rhacophorus* spp.) which often perch on leaves. Aquatic species (including tadpoles) will also been shown to benefit from refugia. Ensure that any live plants are obtained from sources free from agrochemicals. Also make sure that plants do not have thorns, irritant or toxic sap, hairy leaves/stems or other features that may harm amphibians. Additionally, food insects may feed on plants in terraria before being consumed, so ensure that plant toxins cannot be ingested by amphibians by this route.

Light and temperature

Wild amphibians have evolved in accordance with their environments. They will reconcile requirement and provision by self-regulating their exposure to heat, light and the associated UVB radiation. Optimal UVB, light and temperature levels for a species will be those in their natural habitat, specifically at the microhabitat level for each life-history stage. Where possible the UVB, light and temperature microclimate throughout the year should be established through field studies and used to inform the captive management of the target amphibian species. The UVB and Vitamin D3 requirements of amphibians are largely unknown. It is likely that different species will have different requirements due to their differing life histories and microhabitats. UVB is important in the synthesis of vitamin D3. Vitamin D3 deficiency is a nutritional problem encountered in captive amphibians. Vitamin D3 plays a critical role in regulating calcium metabolism, as well as important roles in muscle contraction, organ development and the functioning of the immune and nervous systems. In the majority of vertebrates, vitamin D3 is synthesised via exposure to the ultraviolet B radiation (UVB) present in sunlight. The synthesis of vitamin D3 in the skin requires heat, therefore heat must be provided in tandem with UVB radiation, matching temperatures found in the wild. Some amphibian species might also receive

vitamin D3 through some diets although the extend of this is largely unknown; some food supplements can provide vitamin D3 artificially although great care must be taken to achieve the correct dosages and that the supplement is stored correctly and stock is frequently replaced. Dietary supplements are degraded by light, humidity and heat.

In order to mimic the situation in the wild, heat (infrared radiation), light and UVB should be provided together (i.e. the highest levels of UVB should be associated with the warmest areas of the enclosure, and the lowest levels with the coolest areas). Most importantly, all three must be provided as gradients in the enclosures for appropriate lengths of time each day in order to facilitate self-regulation, with retreats that provide deep shade and no direct UV radiation. If there are life-history stages that would, in the wild, only be exposed to low-level reflected or diffused UV-B, or none at

all (e.g. eggs and tadpoles in tree holes or burrows), then care should be taken to replicate this situation in captivity. The provision of appropriate UV-B radiation to captive amphibians is important for their health and proper development but this field is still in its infancy. We strongly advise caution in its provision as over-exposure can also cause harm. We believe that tolerances to UV-B radiation will be species-specific and related to the historic exposure of animals; individuals that have not been provided with UVB in the past should be slowly exposed to increasing levels until reaching optimal exposure to allow the skin to adapt to radiation.

Amphibians should not be able to come into direct contact with any lamps or heaters as they have extremely sensitive skins and may be easily burnt. All lamps will emit heat but additional heating and cooling may also be necessary. Rooms can be heated and cooled with air conditioning units.

Water quality parameters: guidelines for keeping amphibians. Adapted from Odum & Zippel (2008).

Water quality parameter	Recommended levels	Control methods
Water hardness (dissolved Ca and Mg salts)	For soft water amphibians: <75mg litre ⁻¹ (ppm) of CaCO ₃ . For hard water amphibians: >100mg litre ⁻¹ of CaCO ₃ .	Soft water: Harden using Ca and Mg salts (only recommended reconstituting RO (reverse osmosis), DI (de-ionized) or distilled water). Hard water: Soften using RO, DI or distilled water.
Dissolved oxygen as O ₂ Gas supersaturation	>80% saturation Gases maintained at equilibrium with the atmosphere.	Aeration Aerate water until equilibrium with atmosphere is achieved.
Ammonia/ Ammonium-NH ₃ /NH ⁺ ₄ Nitrites NO ⁻² Nitrates NO ⁻³	<0.2mg litre ⁻¹ , N as unionized ammonia <1.0mg litre ⁻¹ , ideally 0 <50.0mg litre ⁻¹	Biological filtration, chemical filtration (with appropriate medium), water changes. Biological filtration, chemical filtration (with appropriate medium), water changes. Removal: photosynthetic action of green plants or water changes.
pH	Generally near neutral, although it is species-dependant. Should avoid pH <6 and >8.	Change water source or add appropriate buffer solution.
Chlorine Cl ₂	0	Aerate for 24 hours, or add chemical dechlorinator such as sodium thiosulphate.
Chloramines (CINH ₂ , CIN ₂ H, CIN ₃)	<0.01mg litre ⁻¹ as Cl	Use chemical treatment specific for chloramines such as Prime® (Seachem Laboratories, Inc., Madison, GA 30650, USA). Filters for this purpose are available.
Copper (Cu)	<0.05mg litre	Carbon filtering and carbonate precipitation (do not use copper piping).
Phosphates (PO ₃ ⁻⁴)	Toxicity species-specific; EPA recommends limit of 10mg litre ⁻¹ ; 1mg litre ⁻¹ is effective for preventing pipe corrosion.	Lower levels of phosphates using phosphate sponges and filters.

Amphibian enclosures should not be placed under such units, or normal fans, as the air movement can cause severe dehydration. Chilling systems can be installed to cool water in enclosures.

Maximum and minimum temperatures in amphibian enclosures in both the cool end and warm end of the enclosures should be recorded on a daily basis. The UV index should also be recorded to ensure that UVB provision is adequate. The UVI index can be measured using a Solarmeter 6.5.

Water

Water quality is an extremely important component of amphibian husbandry. Monitoring water quality is vital to successfully rearing healthy captive amphibians; fluctuating water parameters create stress for the individuals, therefore it is better to maintain constant conditions, even if these are slightly sub-optimal – of course consistent optimal water quality should always be the goal.

It is important to know certain parameters of the water source; sources can be tested for dissolved substances, pH and hardness, and treated if necessary to provide the appropriate conditions. Unfortunately the optimal water parameters are unknown for most amphibian species. Most institutions use municipal water sources which are generally treated with chlorine, or chloramines; levels of these chemicals should be tested for and treated accordingly. Other water sources should be treated with caution; avoid using water which has previously been in contact with other amphibians as this may risk disease transmission, or may have been contaminated by dissolved substances. Reverse osmosis (RO) systems will produce water that is too pure for amphibians and using pure RO may cause osmoregulatory imbalances. RO water can be made suitable either by adding salts or a known quantity of tap water. Avoid water that has been stored in metal tanks or exposed to any other substances that could leach into the water. Low levels of dissolved metals can be toxic to amphibians.

Water parameters should be tested frequently in newly established systems, and tests can become less frequent as the

system develops. Testing should be carried out on a regular basis in order to monitor the effectiveness of biological filtration.

The most common system used for keeping aquatic and semi-aquatic animals is a semi-closed system, in which a combination of filtration and water changes are employed in order to maintain water quality. Water changes should be small and regular (no more than 20% of the original volume of the water body in question). Rapid changes in water parameters can be highly stressful on the physiology of aquatic organisms. The necessary frequency of water changes will depend on the stocking density, the temperature of the water and the amount and type of food given at each feed, as these have an effect on the level of activity within the tank, which in turn affects the amount and character of waste material produced.

Suspended particles can be removed from the water column through mechanical filtration, dissolved harmful substances (such as chlorine) through chemical filtration, and nitrogenous waste material produced by aquatic organisms and the breakdown of organic material can be processed by bacteria living on filter media, through the process of biological filtration. This can be achieved through using separate filters, or single filters with combined functions. The outflow of the filter should be adjusted to meet the requirements of the amphibian. Some tadpoles require extremely fast flowing well oxygenated water whereas this may be detrimental to other tadpoles (e.g. those living in ponds).

The size and type of filter used will depend on the size of the enclosure, volume of water and density of individuals being catered for. If larvae are being kept at relatively high densities, large external filters may be necessary to remove waste material; for those kept at lower densities (under 20 individuals per litre), box filters may be adequate. Filters require regular maintenance in order to ensure they continue to function effectively. The frequency with which filters need servicing depends on the stocking density, the amount of food given per individual per feed, as well as the temperature of the water, as this will affect how much waste material is produced.

Mechanical filters remove physical particulates from the system, and these then build up in the filter if they are not cleaned out straight away. Build up should be avoided as it will reduce the capacity of the filter for dealing with further suspended particles, and any organic matter within them may begin to break down and leach harmful substances back into the system.

Chemical filters are designed to remove dissolved substances such as organic compounds from the water as it passes through; activated carbon has been identified as an ideal material for amphibian systems and is commonly used as a chemical filtration medium. It is vital that such filter systems are used in combination with a mechanical filter, as build-up of particulate matter will severely compromise their effectiveness. Chemical filter media will need to be routinely replaced. Be aware that activated carbon will remove medication and tannins from the water column, so may be inappropriate for some systems and under some circumstances.

Biological filtration systems consist of a community of living organisms which act to break down toxic metabolic waste products in the water to less toxic substances; toxic ammonia is broken down to less toxic nitrite which then in turn is converted to less toxic nitrate (which in itself is still less toxic to aquatic organisms). These filters are therefore the most important component in maintaining the health of the aquatic system and the amphibians themselves.

Owing to the living component of this filtration system a constant supply and flow of water is required in order to ensure nitrogenous waste is absorbed and that oxygen is available to the bacterial community. Biological filters take time to mature and can be seeded with filter media from established systems, this may risk introducing disease causing agents into new set ups. Alternatively filters can be matured using an ammonium chloride dosing regime. Note that filters are less efficient at processing nitrogenous waste in soft water and at low temperatures.

Consideration must be given to the access points in and out of the water body. Many amphibians are surprisingly poor swimmers and can easily drown.

Plants

Plants are beneficial in amphibian enclosures. Not only do they provide resting sites for amphibians but they also create microhabitats (e.g. humid pockets of air caused by transpiration and also shade from heat and light) as well as oviposition sites for some amphibian species. Plants are particularly important in aquatic exhibits as they will use the nitrogenous compounds for growth, as well as absorbing many other pollutants, and can improve water quality.

Amphibian house exhibiting various species.



Diet

Nutritional problems are a major barrier to successful amphibian husbandry. The nutritional requirements of most amphibians are unknown, and requirements change with life stage for species with tadpoles.

Even when the diet is known, it is often impossible to replicate in captivity as captive diets are limited by the commercial availability of food species and the ability to establish breeding colonies of appropriate species, as well as difficulties in providing prey species themselves with suitable diets. Nevertheless, nutrition is a key factor in keeping amphibians healthy and a basis for successful breeding. Great efforts should be made to be able to provide any captive amphibians with as much variety and good nutritional content as possible.

Most post metamorphic amphibians are insectivores and will only feed on live, moving prey items. Invertebrates can be cultured or collected from the field. Food offered to amphibians should be no bigger than the width of the head. All food should be well fed prior to being offered to the amphibian, in addition to being dusted with an appropriate dietary supplement. To encourage natural feeding behaviour amphibians should be fed at times to

coincide with their peak activity periods to ensure that they encounter the food and that dietary supplements are still coating the prey item when the food is ingested.

Seasonality and breeding

Many amphibians breed in response to environmental cues, these include, but are not limited to temperature, humidity, precipitation, barometric pressure and changes in water quality. It is important that seasonal changes that reflect the situation in nature are replicated in captivity even if breeding is not the desired outcome as failing to provide animals with the seasonality may cause health issues e.g. females becoming egg bound, obesity etc.

Rain chambers are one of the most common ways to breed amphibians in captivity. These systems recreate rain showers and usually involve a small pump sitting in a few centimetres of water, the pump supplies water to a rain bar. The frequency and duration of the rains can be controlled by using a timer.

Routine maintenance

Faecal material and dead feeder insects should be removed from enclosures on a daily basis. Any uneaten food should be removed the day after the animals have

Rearing tadpoles can be an easy way for ex-situ conservation



been fed as the nutritional value of the food will have deteriorated during the time it is has been in the amphibians' enclosure. Feeding regimes will depend on the species being maintained, the season and life stage of the amphibian. Water dishes should be changed daily and the same attention should be paid to the water source for water dishes as for aquaria. Amphibians often excrete large volumes of urine whilst sitting in water and even if the water appears clean it may not be. Captive amphibians (particularly non-native species) may carry pathogens that are not present in the vicinity of the amphibian holding facility. Waste water from amphibian enclosures should be disinfected following the manufacturer's guidelines. Temperatures should be checked and recorded daily and the function of all aquatic life support systems lights and heaters checked on a daily basis. Special attention should be paid to humidity gradients within the enclosures, if an area has become too dry it can be manually sprayed with water from an appropriate source. Ideally amphibians should be given a visual inspection on a daily basis. Unearthing fossorial species such as caecilians and some frogs may be detrimental to their health and welfare so disturbance should be minimised for these species.

Following is the list of target species and practice species with the name of zoological parks identified by the Central Zoo Authority (Ministry of Environment, Forest & Climate Change, Government of India) during the year 2013 with an objective to initiating the amphibian housing, exhibit and if required planned conservation breeding in India".



Target Species

1. *Nasikabatrachus sahyadrensis* (WG)
2. *Melanobatrachus indicus* (WG)
3. *Tylototriton verrucosus* (NE)
4. *Scutiger occidentalis* (H)
5. *Indirana phrynoderma* (WG)
6. *Pterorana khare* (NE)
7. *Rhacophorus tuberculatus* (NE)
8. *Ingerana charlesdarwini* (AN)
9. *Bufo hololius* (Penninsular)
10. *Microhyla sholigari* (Penninsular)
11. *Bufoides meghalayanus* (NE)
12. *Rhacopharous pseudomalabaricus* (WG)
13. *Bufoides kempii* (NE)
14. *Pedostibes tuberculosus* (WG)
15. *Polypedates insularis* (AN)
16. *Nyctibatrachus vasanthi* (WG)
17. *Raorchestes chalazodes* (WG)

Zoological Parks identified by the Central Zoo Authority (Ministry of Environment, Forest & Climate Change, Government of India) for ex situ management of amphibians:

1. Arignar Anna Zoological Park, Vandalur, Chennai

- a) *Euphyctis cyanophlyctis*
- b) *Duttaphrynus melanostictus*
- c) *Ramanella variegata*
- d) *Polypedates maculatus*

2. Nandankanan Biological Park, Bhubaneswar

- a) *Sphaerotheca breviceps*
- b) *Polypedates maculatus*
- c) *Duttaphrynus melanostictus*
- d) *Kaloula pulchra*

Rearing of insects to feed amphibians in captivity.

3. Madras Crocodile Bank Trust, Mamallapuram

- a) *Duttaphrynus melanostictus*
- b) *Sphaerotheca rolandae*
- c) *Polypedates maculatus*
- d) *Ramanella variegata*

4. Chennai Snake Park, Chennai

- a) *Uperodon systoma* (All India)
- b) *Fejervarya limnocharis*
- c) *Duttaphrynus melanostictus*

5. Rajiv Gandhi Zoological Park, Pune

- a) *Microhyla ornata*
- b) *Duttaphrynus melanostictus*
- c) *Polypedates maculatus*

6. Padmaja Naidu Himalayan Zoological Park, Darjeeling

- a) *Tylotriton himalayanus*
- b) *Duttaphrynus himalayanus*
- c) *Ichthyophis sikkimensis*

7. Pilikula Biological Park, Mangalore

- a) *Hylarana malabarica*
- b) *Indirana brachytarsus*
- c) *Ramanella montana*
- d) *Gegeneophis carnosus*

8. Biological Park, Itanagar

- a) *Polypedates leucomystax*
- b) *Clinotarsus alticola*
- c) *Leptobrachium smithi*
- d) *Rhacophorus maximus*

9. Nehru Zoological Park, Hyderabad

- a) *Duttaphrynus melanostictus*
- b) *Sphaerotheca breviceps*
- c) *Polypedates maculatus*

10. Sri Chamarajendra Zoological Gardens, Mysore

- a) *Hylarana malabarica*
- b) *Clinotarsus curtipes*
- c) *Microhyla rubra*
- d) *Ichthyophis beddomi*

11. V.J.B. Udyan Zoo, Byculla Mumbai

- a) *Raorchestes bombayensis*
- b) *Sphaerotheca breviceps*
- c) *Indirana leithii*

12. Bahinabhai Prani Sangrahalaya, Pimpri, Pune

- a) *Rhacophorus malabaricus*
- b) *Raorchestes bombayensis*
- c) *Hylarana malabarica*

13. National Zoological Park, Delhi

- a) *Fejervarya limnocharis*
- b) *Duttaphrynus stomaticus*
- c) *Polypedates maculatus*

14. M.C. Zoological Park, Chhatbir, Chandigarh

- a) *Chiromentis dudhwaensis*
- b) *Fejervarya limnocharis*
- c) *Duttaphrynus stomaticus*

15. Tata Steel Zoological Park, Jamshedpur

- a) *Duttaphrynus stomaticus*
- b) *Uperodon systoma*
- c) *Polypedates maculatus*
- d) *Sphaerotheca dobsoni*

16. Bhagwan Birsa Zoological Park, Ranchi

- a) *Duttaphrynus stomaticus*
- b) *Uperodon systoma*
- c) *Polypedates maculatus*
- d) *Sphaerotheca dobsoni*

17. Pt. Govind Ballabh Pant High Altitude Zoo, Nainital

- a) *Duttaphrynus himalayanus*
- b) *Fejervarya limnocharis*
- c) *Nanorana vicina*

18. Assam State Zoo & Botanical Garden, Guwahati

- a) *Chiromentis simus*
- b) *Kaloula pulchra*
- c) *Fejervarya limnocharis*
- d) *Duttaphrynus stomaticus*
- e) *Polypedates leucomystax*

19. Seppaijala Zoological Park, Sepahijala

- a) *Kaloula pulchra*
- b) *Fejervarya limnocharis*
- c) *Duttaphrynus stomaticus*
- d) *Polypedates leucomystax*

20. Aizwal Zoo, Aizwal

- a) *Kaloula pulchra*
- b) *Fejervarya limnocharis*
- c) *Duttaphrynus stomaticus*
- d) *Rhacophorus maximus*

21. Manipur Zoological Park, Imphal

- a) *Ingerana borealis*
- b) *Duttaphrynus stomaticus*
- c) *Polypedates leucomystax*

22. Biological Park, Chidiyatapu, Andaman & Nicobar Islands

- a) *Ingerana charlesdarwini*
- b) *Micryletta inornata*

23. Laboratory for Conservation of Endangered Species (LaCONES), Hyderabad

- a) *Duttaphrynus melanostictus*
- b) *Sphaerotheca breviceps*
- c) *Polypedates maculatus*

WL- Western Ghats; NE- North East; H- Himalayan; AN – Andaman & Nicobar Islands

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Potential flagship species for improving support and garnering attention towards amphibian conservation in the Western Ghats, India

Abstract

Amphibians are the most threatened vertebrate group in the world as a result of habitat loss, disease, and climate change. In the Western Ghats region, part of the Western Ghats – Sri Lanka biodiversity hotspot in India, amphibians exhibit the highest endemism and are one of the most imperilled vertebrate groups. However, amphibian receive very little conservation attention since the official focus has been on conserving charismatic mega-fauna. To improve this issue of neglect and garner support for amphibian conservation, we initiated the identification of 'flagship' amphibian species which would appeal to stakeholders (local communities, conservation practitioners and tourists) and initiate positive conservation action. By using different levels of eight criteria, viz, recognition, status, distribution, visibility, appearance, unique characteristics, local significance, and media coverage, we identified 46 potential flagship species from the 229 amphibians known from the Western Ghats region. Of the 46 species: *Rhacophorus pseudomalabaricus*, *Nasikabatrachus sahyadrensis*, *Rhacophorus lateralis*, *Xanthophryne tigerina*, *Ghatixalus variabilis* and *Raorchestes chlorosomma* were potential flagships for stakeholders. We recommend piloting the potential flagship species on the ground to ascertain their effectiveness before their use in conservation programs and campaigns.

Introduction

Amphibians are one of the most threatened vertebrate groups with close to a third of the species facing a heightened risk of extinction (Hoffman et al. 2010; IUCN 2017; Stuart et al. 2004). As a group, they face severe population declines, ongoing local extirpations and global extinctions due to a wide array of threats ranging from climate change, habitat loss, and disease (Pounds et al. 2006; Skerratt et al. 2007; Sodhi et al. 2008).

Among vertebrates in the Western Ghats, amphibians exhibit the highest endemism (Myers et al. 2000). As of January 2017, 229 species of amphibians are known from the Western Ghats, of which 62 are threatened (IUCN 2017; Appendix 1). Amphibians in the Western Ghats region of the Western Ghats - Sri Lanka biodiversity hotspot face challenges similar to amphibians on the

Key words: anurans, caecilians, conservation practitioner, frogs, local communities, marketing, stakeholders, tourist, Western Ghats - Sri Lanka Biodiversity Hotspot

Melanobatrachus indicus Black
Narrow Mouth Frog, Kalakad
Mundanthurai Tiger Reserve.
Photo Credit: Varun Kher

**Arun Kanagavel^{1,5,*}, Lilly Margaret Eluvathingal²,
Sethu Parvathy^{1,5}, Ramachandran Kotharambath³
& Sandeep Das⁴**

¹Conservation Research Group, St. Albert's College, Banerji Road, Kochi 682 018, India

²Florida International University, Miami, Florida, 33199, USA

³Department of Animal Science, Central University of Kerala, Kasaragod, Kerala, India

⁴Forest Ecology and Biodiversity Conservation Division, Kerala Forest Research Institute, Peechi, Kerala, India

⁵Women in Conservation and Climate Action Network, 4D, Skyline Melody Apartment Vazuthacaud, Thiruvananthapuram, 695014, Kerala, India

*Corresponding author e-mail: arun.kanagavel@gmail.com



global scale -habitat loss and deterioration, habitat fragmentation, dams, and chemical pollution (Daniels 1991; Gurushankara et al. 2007; Kumar et al. 2002; Naniwadekar & Vasudevan 2014). In addition, frog meat is also consumed locally and is used in traditional medicine (Kanagavel et al. 2016; Thomas & Biju 2016). Local myths about amphibians have led to reduced local support for amphibian conservation and at times results in their culling (Harpalani et al. 2015; Kanagavel et al. 2017; Kotharambath et al. 2013). Despite these threats and high endemism, amphibians receive very little conservation attention from local and national stakeholders. This is especially true since the 'official focus' is on charismatic large mammals like the Bengal tiger (*Panthera tigris tigris*) and Asian elephant (*Elephas maximus*; WII-ENVIS 2017). There is an urgent need to initiate on-ground conservation initiatives at least for highly threatened and endemic amphibians, since unlike mammals they mostly cannot disperse over large distances, are extremely sensitive to climate/habitat change, and occupy highly restricted ranges (Smith & Green 2005; Sodhi et al. 2008).

To highlight the case of amphibians in the Western Ghats, representatives from the 229 species need to be carefully selected, to serve as 'flagships' for the entire group and positively influence stakeholders. A flagship

species is "a species used as the focus of a broader conservation marketing campaign based on its possession of one or more traits that appeal to the target audience" which can vary depending on the conservation issue to be mitigated (Verissimo et al. 2011). This study aims to identify potential flagship amphibian species in the Western Ghats of India that would help in building appreciation towards this vertebrate group, improve local support, and focus on-ground conservation.

Methods

A list of amphibian species (anurans and caecilians) from the Western Ghats of India was compiled from existing checklists (Dinesh et al. 2015) and with new species described until January 2017 (Appendix 1). As per Verissimo et al.'s (2011) marketing approach to selecting flagship species, we first identified lack of conservation attention, support, and appreciation as the conservation problems to be tackled. The target audiences selected were three different stakeholders: local communities, tourists, and conservation practitioners. In this study, local communities refer to those individuals living in and around the habitats of amphibians. Tourists refer to those individuals who not only visit forested areas for recreation but also individuals in urban settlements far away from the amphibian

Figure 1: Anamalai gliding frog
Rhacophorus pseudomalabaricus
Photo Credit: Sandeep Das



habitats. Conservation practitioners include forest department officials, related government institutions, non-governmental organizations, and researchers. Different flagship species were identified for different stakeholders, as they are known to have different preferences with respect to the conservation issue to be mitigated and, campaigns including the selection of flagship species need to be formulated accordingly (Kanagavel et al. 2014; Verissimo et al. 2011).

In accordance to the next step of the marketing approach, we identified eight criteria from the existing literature on flagship species to assist in identifying potential flagship amphibians in the Western Ghats. These criteria were selected based on data availability, and our perception of whether it was applicable for amphibians in the Western Ghats taking in to consideration the different stakeholders (Bowen-Jones & Entwistle 2002; Smith et al. 2012; Verissimo et al. 2009, 2014). Recognition or distinctiveness (Bowen-Jones & Entwistle 2002) was chosen as a criterion so that the flagship species chosen are easily distinguishable and not confused with other species in the locality by the stakeholders. The IUCN threat status was chosen as a criterion specifically for conservation practitioners as they are more concerned about threatened species (Home et al. 2009). The Wildlife Protection Act, 1972 was not considered for this criterion as it provides an insufficient list of amphibian species (WPA 1972). Currently, it only mentions "Fresh Water Frogs (*Rana* spp.)" under Schedule IV, which is inappropriate since nearly all amphibians are freshwater species and the taxonomy of this vertebrate group has changed vastly during the last 40 years. Sodhi et al. (2008) recommended that species with restricted ranges should be of higher conservation priority, because of which distribution was chosen as a criterion for conservation practitioners as their objective is to conserve biodiversity. Tourists also tend to prefer endemic species over widespread ones (Verissimo et al. 2009). Visibility, which refers to the possibility of spotting the species in the field (Verissimo et al. 2009, 2014) was chosen for both tourists and local communities, since if the stakeholders were unable to see the species

even after multiple visits to the field, they would lose interest in the species.

Appearance was selected as a criterion for local communities and tourists, as they prefer species that are attractive (see Kanagavel et al. 2014; Verissimo et al. 2009, 2014). This was not used for conservation practitioners, as it is counter-intuitive to their objective of protecting biodiversity biased by appearance. Unique characteristics (e.g. foot flagging behaviour of *Micrixalus* sp. (Biju et al. 2014); parental care in caecilians and *Nyctibatrachus* sp. (Biju et al. 2011; Measey et al. 2003); bird-like call of *Ghatixalus* sp.) for the species was chosen specifically for tourists, as such traits would invoke greater interest in the specific species (Verissimo et al. 2009). Whether a species was locally significant or not, was selected solely for local communities, since it meant that the species would be locally identifiable (Bowen-Jones & Entwistle 2002). This criterion was a combination of various local community related criteria listed by Bowen-Jones & Entwistle (2002), as there is very little information and/or local associations with amphibians in the Western Ghats. Irrespective of whether the local significance of the species was positive or negative, we considered it significant, as 'any publicity is good publicity'. Amphibians are largely 'unknown products' in the Indian biodiversity scenario in comparison to 'established products' like the Bengal tiger and Asian elephant (Sorensen & Rasmussen 2004). Due to the increased 'product' awareness available through negative associations, such species provide an opportunity to engage with local communities, modify their negative associations into positive relationships through conservation initiatives and thereby improve local support for the species and the group. Media coverage was perceived by us to be an important criterion specifically for tourists, as the 'product' if already 'visible' amongst this stakeholder group makes it relatable and cost-effective in garnering greater attention towards the species. Information on these eight criteria detailed in Table 1 were collated from available literature, personal observations of the authors and their colleagues, and the IUCN Red List (IUCN 2017; Table 1).

Table 1: Description of criteria based on which potential amphibian species were identified

Criteria	Description & grouping	Source
Recognition	Whether the species is distinct and can be easily distinguished from other species in the locality	Existing literature, authors personal observations, KV Gururaja (pers. comm.)
Status	Threat status as per IUCN Red List	IUCN 2017
Distribution	Distribution range; classified as point endemic, state endemic or occurring in more than one state. Point endemic includes species, which occupy restricted ranges across adjacent states and a single hill range	IUCN 2017, existing literature, authors personal observations, KV Gururaja (pers. comm.)
Visibility	Refers to the possibility of spotting the species in the field under the assumption that the visit is undertaken during the appropriate season, weather conditions and time period; classified into 25% chance of seeing it during a field visit, 50% or 75%	Existing literature, authors personal observations, KV Gururaja (pers. comm.)
Appearance	Whether the species is visually attractive or not	The perceptions of five different volunteers were averaged to determine whether the species was attractive or not.
Unique characteristics	Whether the species exhibits unique behavioural, ecological, reproductive or vocal characteristics	Existing literature, authors personal observations
Local significance	Whether the species is locally utilised, has local beliefs attached to it or is distinctly recognised by communities	Existing literature, authors personal observations, KV Gururaja (pers. comm.)
Media coverage	Whether the species has been significantly mentioned (beyond the mention of species name and location) in newspapers, local magazines and online news portals.	Online searches, newspapers and magazines

Data analysis

Only species that were morphologically distinct were selected to avoid any confusion with other species in the same locality. Potential flagship species were then chosen based on criteria appropriate for each stakeholder as previously detailed. The species that performed the best among the chosen criteria were ranked and selected as potential flagship species. For local communities, those species that either fulfilled all the criteria (appearance = attractive, local significance = yes, visibility = 75/50; Ranking = 1) or all but one criteria (only 75% visibility was applicable; Ranking = 2) were selected. For tourists, the species that either fulfilled all the criteria (distribution = point endemic/state endemic, appearance = attractive, media coverage = yes, unique characteristics = yes, visibility = 75/50; Ranking = 1), or all but one criteria whose visibility was 75% (Ranking = 2), or all but one criteria whose visibility was 50% (Ranking = 3), or all but two criteria (only 75% visibility was

applicable; Ranking = 4) were chosen. For conservation practitioners, the species that were Critically Endangered and were designated point endemics (Ranking = 1) or those that were Endangered and point/state endemics (Ranking = 2) were chosen. This selection process was designed as such to select the best potential flagship species. The lower the ranking the higher is the potential of the species to perform well as a flagship. We did not ground-truth the effectiveness of the identified flagship species on the ground as per the final step of the marketing approach to select such species. Due to this we term the species identified in this manner as potential flagship species.

Results and Discussion

While there has always been interest in the conservation of charismatic mega-fauna in India, smaller vertebrates like amphibians and freshwater fish are yet to receive their fair share of attention (Robin & Nandini 2012). A total of 46 amphibians including a caecilian species were identified as



potential flagship species in building appreciation towards amphibians, improving local support, and increasing on-ground conservation in the Western Ghats (Table 2). Nineteen flagship species were identified for local communities, 29 for tourists, and 23 for conservation practitioners (Table 2). Six species, *Rhacophorus pseudomalabaricus* (Fig. 1), *Nasikabatrachus sahyadrensis* (Fig. 2), *R. lateralis* (Fig. 3), *Xanthophryne tigerina* (Fig. 4), *Ghatixalus variabilis* (Fig. 5), and *Raorchestes chlorosomma* (Fig. 6) were potential flagships for all the stakeholders. *N. sahyadrensis* can be considered as the species which stimulated and inspired amphibian-related research in India, discovery of which received global coverage and attention (Aggarwal 2004). The species is also one of the few that is well known by local communities (Aggarwal 2004; Thomas & Biju 2016). *Rhacophorus pseudomalabaricus* is the only amphibian in recent times to be featured on a postage stamp issued by India and is also locally well known (Harpalani et al. 2015). The other four of the highest performing flagship species are novel and have not been used as flagship species in the past.

We would like to caution conservation practitioners about the existing flux in

anuran taxonomy across the Western Ghats. We recommend that this list be used as a baseline because of the fast pace at which taxonomic revisions are occurring and new species/genera are being described. Even with the current flux in anuran taxonomy, given the high rates of endemism and the threatened status of amphibians in the Western Ghats, it is pertinent to identify flagship species to initiate suitable species-specific and stakeholder-specific conservation programs. The potential flagship species need to be piloted to check whether they are effective for conservation programs and for the target audience before their long-term use in any program/campaign (Verissimo et al. 2011). Moreover, if the scale of the program is changed, to include the entire Indian sub-continent or to focus on a small town in the Western Ghats, flagship species would need to be selected from the amphibian assemblages occurring in the locality.

We present a list of criteria relevant for the amphibians of the Western Ghats region of India, that can be used to determine flagship species for different stakeholders. These selection criteria can be changed based on the conservation issue being mitigated and the characteristics of the audience group. While collating data for the different criteria, we realised that the IUCN

Figure 2: Purple frog
Nasikabatrachus sahyadrensis
Photo Credit: Sandeep Das



Figure 3: Boulenger's Tree Frog
Rhacophorus lateralis
Photo Credit: Sandeep Das

Red List assessment needed to be updated for numerous species based on current scientific literature, and assessments needed to be undertaken for several newly described species. The resulting flagship species for conservation practitioners would be different if the assessments were up to date. We suggest that a quicker online channel be setup for researchers to modify or add new IUCN Red List assessments in collaboration with the regional chair of the IUCN SSC Amphibian Specialist Group. The schedules of Wildlife Protection Act, 1972 must be updated, reflecting the current taxonomic status, threat status and trade of amphibians, which would not only be an invaluable source for such prioritizations but also for enhancing amphibian conservation in India. When new species are being described, we suggest that species association with local communities also be investigated and mentioned in research literature. Field studies could also collect such information from local communities as there is a severe lack of information regarding the local significance/associations with amphibians. If investigated, it could reveal species with local significance (Harpalani et al. 2015; Kanagavel et al. 2017, Turvey et al. 2015), which will be effective for conservation programs with local communities. We also observed an exceptional rise in media coverage for recently discovered species and suggest that these articles include more

about the species beyond mentioning its name and locality. Official nature-based tourism organized by the Forest Department does not integrate amphibians as it mainly involves mammals and birds, especially since access to forest areas is allowed only between 06:00 to 18:00 hr. Official programs that provide an opportunity to observe and research the appropriate flagship species in the wild could improve appreciation of amphibians among urban communities, generate financial support for the Forest Department to improve amphibian conservation and support local livelihoods if designed as a community-based initiative. This effort to identify appropriate flagship amphibian species is only the beginning and we encourage the community to help make it more informative and updated.

Figure 4: Amboli Toad
Xanthophryne tigerina
Photo Credit: Varad B. Giri





Figure 5: Star-eyed Tree Frog
Ghatixalus variabilis
Photo Credit: Sandeep Das



Figure 6: Green-eyed Bush Frog
Raorchestes chlorosomma
Photo Credit: Sandeep Das

Table 2: Potential flagship amphibians of the Western Ghats

	Scientific Name	Local Community*	Tourist*	Conservation Practitioner*
1	<i>Rhacophorus pseudomalabaricus</i>	1	1	1
2	<i>Nasikabatrachus sahyadrensis</i>	1	1	2
3	<i>Rhacophorus lateralis</i>	1	2	2
4	<i>Xanthophryne tigerina</i>	2	2	1
5	<i>Ghatixalus variabilis</i>	2	2	2
6	<i>Raorchestes chlorosomma</i>	2	4	1
7	<i>Raorchestes chalazodes</i>	-	1	1
8	<i>Rhacophorus malabaricus</i>	1	2	-
9	<i>Beddomixalus bijui</i>	2	2	-
10	<i>Ghatixalus asterops</i>	2	2	-
11	<i>Raorchestes resplendens</i>	-	3	1
12	<i>Uperodon taprobanica</i>	1	4	-
13	<i>Micrixalus adonis</i>	3	2	-
14	<i>Raorchestes nerostagona</i>	-	3	2
15	<i>Raorchestes travancoricus</i>	-	3	2
16	<i>Duttaphrynus beddomii</i>	-	4	2
17	<i>Raorchestes luteolus</i>	2	4	-
18	<i>Xanthophryne koynayensis</i>	-	4	2
19	<i>Sallywalkerana diplosticta</i>	-	4	2
20	<i>Ichthyophis bombayensis</i>	1	-	-
21	<i>Clinotarsus curtipes</i>	1	-	-
22	<i>Raorchestes ponmudi</i>	-	-	1
23	<i>Duttaphrynus parietalis</i>	2	-	-
24	<i>Ghatophryne ornata</i>	-	-	2
25	<i>Pedostibes tuberculosus</i>	-	-	2
26	<i>Euphlyctis hexadactylus</i>	1	-	-
27	<i>Hoplobatrachus tigerinus</i>	2	-	-
28	<i>Minervarya sahyadris</i>	-	-	2
29	<i>Micrixalus gadgili</i>	-	-	2
30	<i>Melanobatrachus indicus</i>	-	-	2
31	<i>Microhyla rubra</i>	2	-	-
32	<i>Microhyla sholigari</i>	-	-	2
33	<i>Uperodon variegata</i>	2	-	-
34	<i>Raorchestes signatus</i>	-	-	2
35	<i>Raorchestes tinniens</i>	-	-	2
36	<i>Rhacophorus calcadensis</i>	-	-	2
37	<i>Raorchestes manohari</i>	-	3	-
38	<i>Raorchestes ochlandrae</i>	-	3	-
39	<i>Raorchestes uthamani</i>	-	3	-
40	<i>Micrixalus phyllophilus</i>	-	4	-
41	<i>Micrixalus thampii</i>	-	4	-
42	<i>Nyctibatrachus grandis</i>	-	4	-
43	<i>Nyctibatrachus minimus</i>	-	4	-
44	<i>Indirana bhadrai</i>	-	4	-
45	<i>Mercurana myristicapalustris</i>	-	4	-
46	<i>Raorchestes flaviocularis</i>	-	4	-

*Refer to the analysis section for an understanding of the ranking scheme followed for each stakeholder. The lower the ranking, the higher is the flagship potential of the species. This '-' means that the species is not a flagship for the associated stakeholder



Koyna Wildlife Sanctuary, northern western ghats, a key site for amphibian conservation.
Photo Credit: Preeti Sharma

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Appendix 1. Detailed characteristics of the amphibians of Western Ghats based on the eight criteria used to identify potential flagship species (see Table 1 for further details on the eight criteria)

	Species	Recognition	Status ^a	Distribution ^b
	Family: Bufonidae			
1	<i>Duttaphrynus beddomii</i> *	Yes	EN	PE
2	<i>Duttaphrynus brevirostris</i>	No	DD	PE
3	<i>Duttaphrynus melanostictus</i>	No	LC	MS
4	<i>Duttaphrynus microtympanum</i>	Yes	VU	MS
5	<i>Duttaphrynus parietalis</i> *	Yes	NT	MS
6	<i>Duttaphrynus scaber</i>	Yes	LC	MS
7	<i>Duttaphrynus silentvalleyensis</i>	No	DD	PE
8	<i>Duttaphrynus stomaticus</i>	No	LC	MS
9	<i>Ghatophryne ornata</i> *	Yes	EN	PE
10	<i>Ghatophryne rubigina</i>	Yes	VU	PE
11	<i>Pedostibes tuberculosus</i> *	Yes	EN	PE
12	<i>Xanthophryne koynayensis</i> *	Yes	EN	PE
13	<i>Xanthophryne tigerina</i> **	Yes	CR	PE
	Family: Dicroglossidae			
14	<i>Euphlyctis mudigere</i>	No	NE	PE
15	<i>Euphlyctis aloysii</i>	Yes	NE	MS
16	<i>Euphlyctis cyanophlyctis</i>	Yes	LC	MS
17	<i>Euphlyctis hexadactylus</i> *	Yes	LC	MS
18	<i>Hoplobatrachus crassus</i>	Yes	LC	MS
19	<i>Hoplobatrachus tigerinus</i> *	Yes	LC	MS
20	<i>Sphaerotheca breviceps</i>	No	LC	MS
21	<i>Sphaerotheca dobsonii</i>	No	LC	MS
22	<i>Sphaerotheca leucorhynchus</i>	No	DD	PE
23	<i>Sphaerotheca rolandae</i>	No	LC	MS
24	<i>Fejervarya brevipalmata</i>	No	DD	MS
25	<i>Fejervarya caperata</i>	No	NE	PE
26	<i>Fejervarya gomantaki</i>	No	NE	PE
27	<i>Fejervarya granosa</i>	No	NE	MS
28	<i>Fejervarya keralensis</i>	Yes	LC	MS
29	<i>Fejervarya kudremukhensis</i>	No	NE	SE
30	<i>Fejervarya modestus</i>	No	NE	PE
31	<i>Fejervarya mudduraja</i>	No	NE	PE
32	<i>Fejervarya murthii</i>	No	CR	SE
33	<i>Fejervarya mysorensis</i>	No	DD	PE
34	<i>Fejervarya nilagirica</i>	No	EN	PE
35	<i>Fejervarya parambikulamana</i>	No	DD	PE
36	<i>Fejervarya rufescens</i>	Yes	LC	MS
37	<i>Minervarya sahyadris</i> *	Yes	EN	PE
38	<i>Fejervarya sauriceps</i>	Yes	DD	PE
39	<i>Fejervarya syhadrensis</i>	No	LC	MS
	Family: Micrixalidae			
40	<i>Micrixalus adonis</i> *	Yes	NE	PE
41	<i>Micrixalus candidus</i>	Yes	NE	PE
42	<i>Micrixalus elegans</i>	Yes	DD	PE
43	<i>Micrixalus frigidus</i>	No	NE	PE

<i>Visibility</i>	<i>Appearance</i>	<i>Unique characteristics</i>	<i>Local significance</i>	<i>Media coverage</i>
75	No	No	No	Yes
75	No	No	Yes	No
75	No	No	Yes	Yes
50	No	No	Yes	No
75	No	No	Yes	No
50	No	No	Yes	No
50	No	No	No	No
50	No	No	No	No
50	Yes	No	No	No
25	Yes	No	No	No
50	No	Yes	No	Yes
75	No	Yes	No	No
75	Yes	Yes	No	No
75	No	No	No	Yes
75	No	No	No	Yes
75	No	No	No	No
75	Yes	No	Yes	No
50	No	Yes	Yes	Yes
75	No	No	Yes	Yes
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
25	No	No	No	No
75	No	No	No	No
25	No	No	No	No
25	No	No	No	No
50	No	No	No	No
50	No	No	No	No
25	No	No	No	No
50	No	No	No	No
25	No	No	No	No
75	No	No	No	No
50	No	No	No	No
75	No	No	No	No
25	No	No	No	No
75	Yes	Yes	No	No
50	No	Yes	No	No
50	No	Yes	No	No
75	No	Yes	No	No

	Species	Recognition	Status ^a	Distribution ^b
	Family: Bufonidae			
44	<i>Micrixalus fuscus</i>	No	NT	PE
45	<i>Micrixalus gadgili</i> *	Yes	EN	PE
46	<i>Micrixalus herrei</i>	Yes	NE	MS
47	<i>Micrixalus kodayari</i>	No	NE	PE
48	<i>Micrixalus kottigeharensis</i>	No	CR	PE
49	<i>Micrixalus kurichiyari</i>	No	NE	PE
50	<i>Micrixalus mallani</i>	No	NE	PE
51	<i>Micrixalus nellyampathi</i>	No	NE	PE
52	<i>Micrixalus nigraventris</i>	No	NE	PE
53	<i>Micrixalus niluvasei</i>	No	NE	PE
54	<i>Micrixalus nudis</i>	Yes	VU	PE
55	<i>Micrixalus phyllophilus</i> *	Yes	VU	PE
56	<i>Micrixalus sairandhri</i>	No	NE	PE
57	<i>Micrixalus sali</i>	Yes	NE	PE
58	<i>Micrixalus saxicola</i>	No	NE	MS
59	<i>Micrixalus silvaticus</i>	No	DD	PE
60	<i>Micrixalus specca</i>	No	NE	PE
61	<i>Micrixalus spelunca</i>	No	NE	PE
62	<i>Micrixalus thampii</i> *	Yes	DD	PE
63	<i>Micrixalus uttaraghaati</i>	Yes	NE	MS
	Family: Microhylidae			
64	<i>Melanobatrachus indicus</i> *	Yes	EN	PE
65	<i>Microhyla ornata</i>	No	LC	MS
66	<i>Microhyla rubra</i> *	Yes	LC	MS
67	<i>Microhyla sholigari</i> *	Yes	EN	PE
68	<i>Uperodon anamalaiensis</i>	Yes	DD	PE
69	<i>Uperodon minor</i>	No	DD	PE
70	<i>Uperodon montana</i>	No	NT	MS
71	<i>Uperodon mormorata</i>	No	EN	MS
72	<i>Uperodon taprobanica</i> *	Yes	LC	MS
73	<i>Uperodon triangularis</i>	Yes	VU	MS
74	<i>Uperodon variegata</i> *	Yes	LC	MS
75	<i>Uperodon globulosus</i>	Yes	LC	MS
76	<i>Uperodon systoma</i>	Yes	LC	MS
	Family: Nasikabatrachidae			
77	<i>Nasikabatrachus sahyadrensis</i> * *	Yes	EN	SE
	Family: Nyctibatrachidae			
78	<i>Nyctibatrachus acanthodermis</i>	Yes	NE	PE
79	<i>Nyctibatrachus aliciae</i>	No	EN	PE
80	<i>Nyctibatrachus anamallaiensis</i>	No	NE	PE
81	<i>Nyctibatrachus beddomii</i>	No	EN	PE
82	<i>Nyctibatrachus danieli</i>	No	NE	PE
83	<i>Nyctibatrachus dattatreyaensis</i>	No	CR	PE
84	<i>Nyctibatrachus deccanensis</i>	No	VU	PE
85	<i>Nyctibatrachus deveni</i>	No	NE	PE
86	<i>Nyctibatrachus gavi</i>	Yes	NE	PE
87	<i>Nyctibatrachus grandis</i> *	Yes	NE	PE
88	<i>Nyctibatrachus humayuni</i>	No	VU	SE

<i>Visibility</i>	<i>Appearance</i>	<i>Unique characteristics</i>	<i>Local significance</i>	<i>Media coverage</i>
75	No	Yes	No	No
50	No	No	No	No
50	No	Yes	No	Yes
50	No	Yes	No	No
75	No	Yes	No	No
50	No	Yes	No	No
50	No	Yes	No	No
75	Yes	Yes	No	No
75	No	No	No	No
50	No	Yes	No	No
50	No	No	No	No
75	No	Yes	No	No
50	No	Yes	No	No
50	No	No	No	No
75	Yes	Yes	No	Yes
75	No	Yes	No	No
50	Yes	Yes	No	No
50	No	No	No	No
75	No	Yes	No	No
50	No	Yes	No	No
25	Yes	No	No	No
75	No	No	No	No
75	Yes	No	No	No
75	No	No	No	No
50	No	Yes	No	No
25	No	Yes	No	No
75	No	Yes	No	No
50	No	Yes	No	No
75	Yes	Yes	Yes	No
50	Yes	Yes	No	No
75	Yes	No	No	No
75	No	No	No	No
75	No	No	No	No
50	Yes	Yes	Yes	Yes
50	No	Yes	No	No
50	No	Yes	No	No
75	No	Yes	No	No
75	No	Yes	No	No
50	No	No	No	No
75	No	No	No	No
75	No	No	No	No
75	No	No	No	No
50	No	No	No	No
75	No	Yes	No	No
75	No	Yes	No	Yes

	Species	Recognition	Status ^a	Distribution ^b
	Family: Bufonidae			
89	<i>Nyctibatrachus indraneili</i>	Yes	NE	PE
90	<i>Nyctibatrachus jog</i>	No	NE	PE
91	<i>Nyctibatrachus karnatakaensis</i>	No	EN	PE
92	<i>Nyctibatrachus kempholeyensis</i>	No	DD	PE
93	<i>Nyctibatrachus kumbara</i>	No	NE	PE
94	<i>Nyctibatrachus major</i>	No	VU	MS
95	<i>Nyctibatrachus minimus</i> *	Yes	DD	PE
96	<i>Nyctibatrachus minor</i>	No	EN	PE
97	<i>Nyctibatrachus periyar</i>	No	NE	PE
98	<i>Nyctibatrachus petraeus</i>	No	LC	MS
99	<i>Nyctibatrachus pillaii</i>	No	NE	PE
100	<i>Nyctibatrachus poocha</i>	No	NE	PE
101	<i>Nyctibatrachus sanctipalustris</i>	No	EN	PE
102	<i>Nyctibatrachus shiradi</i>	No	NE	PE
103	<i>Nyctibatrachus sylvaticus</i>	No	DD	PE
104	<i>Nyctibatrachus vasanthi</i>	No	EN	PE
105	<i>Nyctibatrachus vrijeuni</i>	No	NE	PE
	Family: Ranidae			
106	<i>Clinotarsus curtipes</i> *	Yes	NT	MS
107	<i>Hydrophylax bahuvistara</i>	Yes	NE	MS
108	<i>Hydrophylax malabarica</i>	Yes	LC	MS
109	<i>Indosylvirana aurantiaca</i>	Yes	VU	PE
110	<i>Indosylvirana caesari</i>	Yes	NE	PE
111	<i>Indosylvirana doni</i>	Yes	NE	SE
112	<i>Indosylvirana flavescens</i>	Yes	NE	PE
113	<i>Indosylvirana indica</i>	No	NE	PE
114	<i>Indosylvirana intermedius</i>	No	NE	PE
115	<i>Indosylvirana magna</i>	Yes	NE	PE
116	<i>Indosylvirana montanus</i>	Yes	NE	SE
117	<i>Indosylvirana sreeni</i>	Yes	NE	MS
118	<i>Indosylvirana urbis</i>	Yes	NE	PE
	Family: Ranixalidae			
119	<i>Indirana beddomii</i>	Yes	LC	MS
120	<i>Indirana bhadrai</i> *	Yes	NE	PE
121	<i>Indirana brachytarsus</i>	No	EN	MS
122	<i>Indirana chiravasi</i>	Yes	NE	PE
123	<i>Indirana duboisi</i>	Yes	NE	PE
124	<i>Indirana gundia</i>	No	CR	PE
125	<i>Indirana leithii</i>	Yes	VU	PE
126	<i>Indirana paramakri</i>	No	NE	PE
127	<i>Indirana salelkari</i>	No	NE	PE
128	<i>Indirana sarojamma</i>	No	NE	PE
129	<i>Indirana semipalmata</i>	No	LC	MS
130	<i>Indirana tysoni</i>	No	NE	MS
131	<i>Indirana yadera</i>	No	NE	PE
132	<i>Sallywalkerana diplosticta</i> *	Yes	EN	PE
133	<i>Sallywalkerana leptodactyla</i>	No	EN	PE
134	<i>Sallywalkerana phrynoderma</i>	No	CR	PE
	Family: Rhacophoridae			
135	<i>Beddomixalus bijui</i> *	Yes	NE	PE

<i>Visibility</i>	<i>Appearance</i>	<i>Unique characteristics</i>	<i>Local significance</i>	<i>Media coverage</i>
50	No	No	No	No
50	No	Yes	No	No
75	No	No	No	No
75	No	No	No	No
50	No	Yes	No	No
75	No	Yes	No	No
75	No	Yes	No	No
50	No	No	No	Yes
50	No	Yes	No	No
50	No	Yes	No	No
50	No	No	No	No
75	No	Yes	No	No
50	No	No	No	No
25	No	No	No	No
25	No	No	No	No
50	No	No	No	No
75	No	Yes	No	No
75	Yes	No	Yes	No
75	No	No	No	No
75	No	No	No	No
75	No	No	No	No
25	No	No	No	No
50	No	No	No	No
50	No	No	No	No
75	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
75	No	No	No	No
75	No	No	No	No
75	No	Yes	No	No
25	No	Yes	No	Yes
50	No	Yes	No	No
50	No	Yes	No	No
25	No	Yes	No	No
50	No	Yes	No	No
25	No	Yes	No	No
50	No	Yes	No	Yes
25	No	Yes	No	No
50	No	Yes	No	No
75	No	Yes	No	No
50	No	Yes	No	No
50	No	Yes	No	No
75	No	Yes	No	No
50	No	Yes	No	No
50	No	Yes	No	Yes
75	Yes	No	No	Yes

	Species	Recognition	Status ^a	Distribution ^b
	Family: Bufonidae			
136	<i>Ghatixalus asterops</i> *	Yes	DD	PE
137	<i>Ghatixalus magnus</i>	Yes	NE	PE
138	<i>Ghatixalus variabilis</i> **	Yes	EN	PE
139	<i>Mercurana myristicapalustris</i> *	Yes	NE	PE
140	<i>Polypedates maculatus</i>	Yes	LC	MS
141	<i>Polypedates occidentalis</i>	No	DD	PE
142	<i>Polypedates pseudocruciger</i>	No	LC	MS
143	<i>Pseudophilautus amboli</i>	Yes	CR	MS
144	<i>Pseudophilautus kani</i>	No	LC	PE
145	<i>Pseudophilautus wynaadensis</i>	No	EN	PE
146	<i>Raorchestes agasthyaensis</i>	Yes	NE	PE
147	<i>Raorchestes akroparallagi</i>	No	LC	PE
148	<i>Raorchestes anili</i>	No	LC	PE
149	<i>Raorchestes archaeos</i>	No	NE	PE
150	<i>Raorchestes aureus</i>	No	NE	PE
151	<i>Raorchestes beddomii</i>	No	NT	PE
152	<i>Raorchestes blandus</i>	No	NE	PE
153	<i>Raorchestes bobingeri</i>	Yes	VU	PE
154	<i>Raorchestes bombayensis</i>	Yes	VU	PE
155	<i>Raorchestes chalazodes</i> *	Yes	CR	PE
156	<i>Raorchestes charius</i>	No	EN	PE
157	<i>Raorchestes chlorosomma</i> **	Yes	CR	PE
158	<i>Raorchestes chotta</i>	No	DD	PE
159	<i>Raorchestes chromasynchysi</i>	No	VU	PE
160	<i>Raorchestes coonoorensis</i>	No	LC	PE
161	<i>Raorchestes crustai</i>	No	NE	PE
162	<i>Raorchestes dubois</i>	Yes	VU	PE
163	<i>Raorchestes echinatus</i>	No	NE	PE
164	<i>Raorchestes flaviocularis</i> *	Yes	NE	PE
165	<i>Raorchestes flaviventris</i>	Yes	DD	PE
166	<i>Raorchestes ghatei</i>	Yes	NE	PE
167	<i>Raorchestes glandulosus</i>	No	VU	PE
168	<i>Raorchestes graminirupes</i>	No	VU	PE
169	<i>Raorchestes griet</i>	No	CR	PE
170	<i>Raorchestes hassanensis</i>	Yes	NE	PE
171	<i>Raorchestes honnametti</i>	Yes	NE	PE
172	<i>Raorchestes indigo</i>	Yes	NE	PE
173	<i>Raorchestes jayarami</i>	No	NE	PE
174	<i>Raorchestes johnceei</i>	No	NE	PE
175	<i>Raorchestes kadalarensis</i>	Yes	NE	PE
176	<i>Raorchestes kaikatti</i>	No	CR	PE
177	<i>Raorchestes kakachi</i>	No	NE	PE
178	<i>Raorchestes lechiya</i>	Yes	NE	PE
179	<i>Raorchestes leucolatus</i>	No	NE	PE
180	<i>Raorchestes luteolus</i> *	Yes	DD	PE
181	<i>Raorchestes manohari</i> *	Yes	NE	PE
182	<i>Raorchestes marki</i>	No	CR	PE
183	<i>Raorchestes montanus</i>	Yes	NE	PE
184	<i>Raorchestes munnarensis</i>	No	CR	PE
185	<i>Raorchestes nerostagona</i> *	Yes	EN	PE

<i>Visibility</i>	<i>Appearance</i>	<i>Unique characteristics</i>	<i>Local significance</i>	<i>Media coverage</i>
75	Yes	Yes	No	No
50	Yes	No	No	No
75	Yes	Yes	No	No
75	No	No	No	Yes
75	No	No	No	No
75	No	No	No	No
50	No	No	No	No
75	No	No	No	No
75	No	No	No	No
75	No	No	No	No
75	No	No	No	No
75	Yes	No	No	No
75	No	No	No	No
50	No	No	No	No
50	No	No	No	No
75	Yes	No	No	No
50	No	No	No	No
50	Yes	No	No	No
75	No	No	No	No
50	Yes	Yes	No	Yes
75	No	No	No	No
75	Yes	No	No	No
50	No	No	No	No
75	Yes	No	No	No
50	No	No	No	No
50	Yes	Yes	No	No
75	No	No	No	No
50	No	No	No	No
25	Yes	Yes	No	No
50	Yes	No	No	No
50	No	No	No	Yes
75	Yes	No	No	No
75	No	No	No	No
75	No	No	No	No
25	No	No	No	No
50	No	No	No	No
50	Yes	No	No	No
75	Yes	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	Yes
50	No	No	No	No
50	No	No	No	No
75	Yes	No	No	No
50	Yes	Yes	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
50	Yes	Yes	No	No

	Species	Recognition	Status ^a	Distribution ^b
	Family: Bufonidae			
186	<i>Raorchestes ochlandrae</i> *	Yes	DD	PE
187	<i>Raorchestes ponmudi</i> *	Yes	CR	PE
188	<i>Raorchestes primarrumpfi</i>	Yes	NE	PE
189	<i>Raorchestes ravii</i>	No	NE	PE
190	<i>Raorchestes resplendens</i> *	Yes	CR	PE
191	<i>Raorchestes signatus</i> *	Yes	EN	PE
192	<i>Raorchestes silentvalley</i>	Yes	NE	PE
193	<i>Raorchestes sushili</i>	No	CR	PE
194	<i>Raorchestes theuerkaufi</i>	No	NE	PE
195	<i>Raorchestes thodai</i>	No	NE	PE
196	<i>Raorchestes tinniens</i> *	Yes	EN	PE
197	<i>Raorchestes travancoricus</i> *	Yes	EN	PE
198	<i>Raorchestes tuberothumerus</i>	Yes	DD	PE
199	<i>Raorchestes uthamani</i> *	Yes	NE	PE
200	<i>Rhacophorus calcadensis</i> *	Yes	EN	PE
201	<i>Rhacophorus lateralis</i> **	Yes	EN	PE
202	<i>Rhacophorus malabaricus</i> *	Yes	LC	MS
203	<i>Rhacophorus pseudomalabaricus</i> **	Yes	CR	PE
	Family: Ichthyophidae			
204	<i>Ichthyophis beddomei</i>	Yes	LC	MS
205	<i>Ichthyophis bombayensis</i> *	Yes	LC	MS
206	<i>Ichthyophis davidi</i>	No	NE	MS
207	<i>Ichthyophis kodaguensis</i>	No	DD	PE
208	<i>Ichthyophis longicephalus</i>	No	DD	MS
209	<i>Ichthyophis tricolor</i>	No	LC	PE
210	<i>Uraeotyphlus gansi</i>	Yes	DD	PE
211	<i>Uraeotyphlus interruptus</i>	No	DD	PE
212	<i>Uraeotyphlus malabaricus</i>	No	DD	PE
213	<i>Uraeotyphlus menoni</i>	No	DD	PE
214	<i>Uraeotyphlus narayani</i>	No	DD	SE
215	<i>Uraeotyphlus oommeni</i>	No	DD	PE
216	<i>Uraeotyphlus oxyurus</i>	No	DD	PE
	Family: Indotyphlidae			
217	<i>Gegeneophis carnosus</i>	No	DD	PE
218	<i>Gegeneophis danieli</i>	No	DD	MS
219	<i>Gegeneophis goaensis</i>	No	DD	MS
220	<i>Gegeneophis krishni</i>	No	DD	PE
221	<i>Gegeneophis madhavai</i>	No	DD	PE
222	<i>Gegeneophis mhadeiensis</i>	No	DD	MS
223	<i>Gegeneophis pareshi</i>	No	NE	PE
224	<i>Gegeneophis primus</i>	No	NE	PE
225	<i>Gegeneophis ramaswamii</i>	Yes	LC	SE
226	<i>Gegeneophis seshachari</i>	No	DD	SE
227	<i>Gegeneophis tejaswini</i>	No	NE	PE
228	<i>Indotyphlus battersbyi</i>	No	DD	SE
229	<i>Indotyphlus maharashtraensis</i>	No	DD	PE

* Potential flagship species applicable for one or two stakeholders

** Potential flagship species applicable for the three stakeholders

^a CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, LC = Least Concern, DD = Data Deficient, NE = Not Evaluated

^b PE = Point endemic, SE = State endemic, MS = More than 1 state.

<i>Visibility</i>	<i>Appearance</i>	<i>Unique characteristics</i>	<i>Local significance</i>	<i>Media coverage</i>
50	Yes	Yes	No	No
75	No	No	No	No
25	No	No	No	No
50	No	No	No	No
50	Yes	No	No	Yes
50	Yes	No	No	No
50	Yes	No	No	No
50	No	No	No	No
50	No	No	No	No
50	No	No	No	No
75	No	No	No	No
50	Yes	No	No	Yes
75	No	No	No	No
50	Yes	Yes	No	No
50	No	Yes	No	No
75	Yes	Yes	Yes	No
75	Yes	Yes	Yes	Yes
75	Yes	Yes	Yes	Yes
25	Yes	Yes	No	No
25	Yes	Yes	Yes	No
25	Yes	No	No	Yes
25	Yes	No	No	No
25	Yes	No	No	Yes
50	No	Yes	Yes	No
25	Yes	No	No	No
25	No	No	No	No
25	No	No	No	No
25	No	No	No	No
25	Yes	No	No	No
25	No	No	No	No
25	Yes	No	No	No
25	No	No	No	No
25	Yes	No	No	No
25	Yes	No	No	No
25	No	No	No	No
25	No	No	No	No
25	No	No	No	No
25	No	No	No	No
25	No	No	No	No
25	No	No	No	Yes
50	No	Yes	No	No
50	No	Yes	No	Yes
25	Yes	No	No	Yes
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Impact of Roads on Indian amphibians - A review

Abstract

Habitat alterations at landscape level impacts small and declining population paradigm in conservation biology. Roads are major cause of fragmentation and degradation of natural habitats and detrimental to wild flora and fauna worldwide. A highly seasonal activity in amphibians that involves adult migration and juvenile dispersal makes them vulnerable to road related mortality. Anamniotic eggs, semi permeable skin and mating strategies that depend on acoustics makes amphibians particularly sensitive to pollution. Direct and indirect impacts of roads on amphibian population may lead to catastrophic decline and extinction. In Indian context such impacts are poorly known. This article reviews the impacts of road on amphibians from Indian perspective and flags the need for effective mitigation strategies to minimize such loss.

Introduction

Road system, from the earliest times, is one of the strongest indicators of a society's level of development. With the increase in human population, roads became the back bone of economic growth and social development of any country. India, currently represents the second largest road system in the world (ca 5.2 million km), after the United States of America (National Highways Authority of India, <http://www.nhai.org>). On the other hand India holds a unique global position in terms of its biodiversity and is counted among 17 megadiverse countries of the world (Myers et al. 2000). Here development comes in conflict with diversity. Studies show that roads promote paucity of negative effects on biodiversity (Forman & Alexander 1998; Forman et al. 2003; van der Ree et al. 2015), continually creating conflicts of disagreement between human well-being and biodiversity and conservation (Laurance & Balmford 2013; Laurance et al. 2014).

India represents almost 5% of total amphibian diversity of the world with 417 species (376 anurans, 2 Salamanders, 39 caecilians (AmphibiaWeb. 2017,

<http://amphibiaweb.org>) 56 % of Indian amphibians are endemic. Most of the Indian amphibian diversity lies in four of the world's biodiversity hotspots (Myers et al. 2000); Western Ghats and Sri Lanka (Western Ghats), Himalaya (Indian Himalaya), Indo-Burma (parts of North east India) and Sundaland (Nicobar Islands) (Pande & Arora 2014). With the increasing discovery of new amphibian species every year, we are contemporaneously facing ecological crisis of amphibian decline. Causes of amphibian decline such as habitat destruction, introduction of exotic predators and competitors, pollution from pesticides, acid precipitation, increased

Key words:

Roads, Indian amphibians, road mortality, amphibian decline, mitigation

Fig 1. *Duttaphrynus melanostictus* is one of the most frequently killed amphibian on Indian roads.

Preeti Sharma¹ & Abhijit Das²

¹UNESCO C2C, Wildlife Institute of India, Chandrabani, Dehradun- 248001, Uttarakhand, India

²Department of Endangered Species Management, Wildlife Institute of India, Chandrabani, Dehradun- 248001, Uttarakhand, India

*Corresponding author e-mail: preeti19292@gmail.com





Fig 2. Schematic representation of various threats (direct and indirect) to amphibians caused due to linear barriers.

levels of ultraviolet radiation, consumption by humans, and climate change have been documented (Elmberg 1993; Blaustein et al. 1994; Pounds & Crump 1994; Stuart et al. 2004).

Roads are also found to cause decline, local disappearance, unequal sex ratio, growing genetical distance in the vicinity of busy roads and behavioural changes (Oxley et al. 1974; Richardson et al. 1997; Wilkins 1982; DeMaynadier & Hunter 2000; Carr & Fahrig 2001; Vos et al. 2001; Houlahan & Findlay 2003; Andrews & Gibbons 2006) on animal populations.

Amphibians are proved to be the most frequently run over vertebrates on roads in different continents even if their proportion is often underestimated due to low detectability caused by low retention time and small size in comparison with the other taxa (Puky 2006; Puky et al 2007). Vehicular traffic causes negative effects on amphibian density (Fahrig et al. 1995) and the traffic-related mortality is highly detrimental, especially for species with small and declining populations (Spellerberg 1998). Beyond the 'on-road' mortality, the range and severity of indirect impacts of roads far exceed those incurred from direct mortality. Such impacts of linear infrastructures are poorly studied in Indian context and thus, information on impacts of roads on population and species level is hardly known.

Amphibia as a Vulnerable Group

Amphibians are one of the most threatened vertebrate groups on the planet (Hof et al. 2011). Frequently characterized by limited dispersal abilities, strong site fidelity and spatially disjunct breeding habitat, amphibians are suffering massive population decline (Duellman & Trueb 1986; Sinsch 1990; Blaustein et al. 1994; Beebee 1996; Berry 2001; Smith & Green 2005).

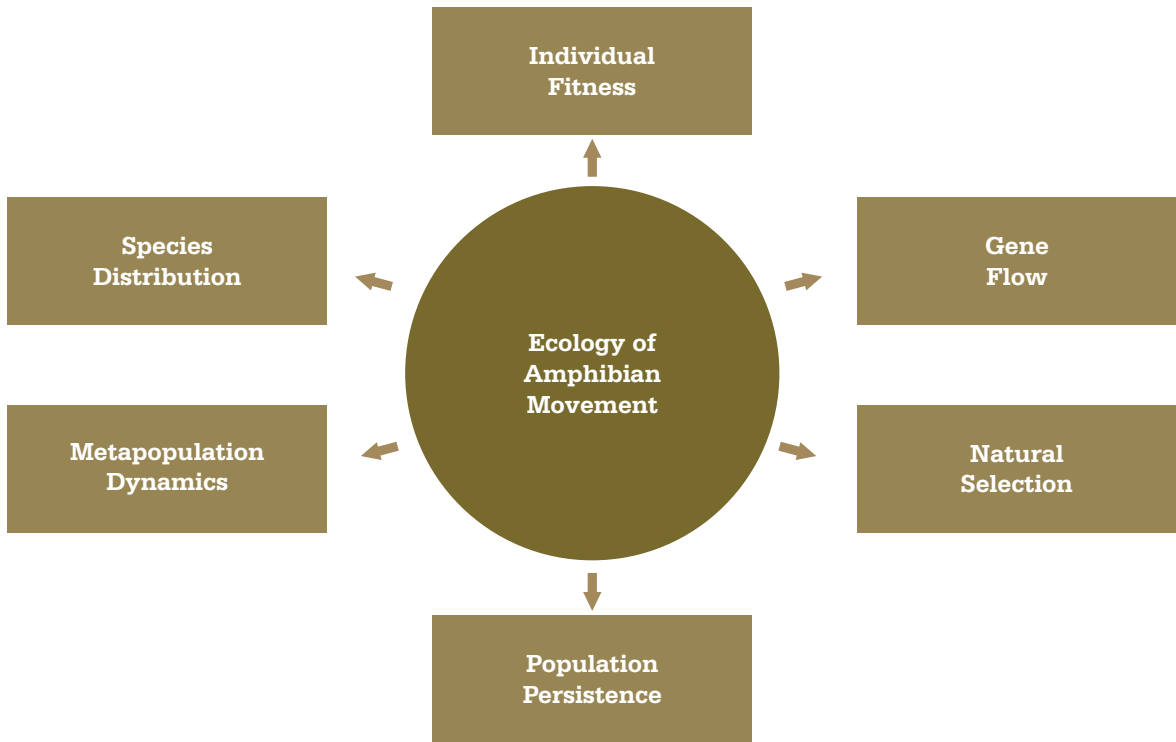
In Ecology, movement is a fundamental need for any species be it a plant or an animal (Nathan et al., 2008; Pittman et al., 2014). Population of any biological species can survive only if individuals can disperse (one directional movement) or migrate (going and coming back). Such spatio-temporal movements thus maintain species distribution, gene flow and individual fitness, which in turn advocate a healthy population dynamics (Clobert et al. 2001; Knowlton & Graham 2010; Mueller et al. 2011).

Physical connectivity between amphibian populations (Stevens & Baguette 2008) is crucial as it plays key role in their regional viability (Cushman 2006). Amphibians appears to be the most vulnerable group from road related impacts as roads not only cut through the human land use types but also slice through most of the ecosystem or habitat types in a landscape (Vermeulen & Opdam, 1995; Puky 2006). Thus, impact of

roads are both direct (vehicular collision), and indirect (fragmentation) causing direct

mortality and/or loss of connectivity between amphibian populations.

Fig 3. Schematic diagram explaining the importance of amphibian movement in a landscape.



Ecological Effect of Roads on Amphibians

Globally, the studies on the ecological effects of roads on wildlife are quite prevalent and expanding rapidly (Nietvelt 2002). However, in India, the field of road ecology is gearing up and is relatively recent (Baskaran & Boominathan 2010; Gubbi et al. 2012; Joshi & Dixit 2012; Prakash 2012; Krishna et al. 2013; Rajvanshi et al. 2013).

Roads pose severe ecological impacts on individual organisms, populations, species, ecosystems and landscapes (Andrews 1990; Bennett 1993; Forman & Alexander 1998; Spellerberg 1998; Trombulak & Frissell 2000; Seiler 2001; Carr et al. 2002; Forman et al. 2003; Coffin 2007).

Road construction leads to the loss, fragmentation, and degradation of herpetofaunal habitats (Trombulak & Frissell 2000, Andrews et al. 2008), and thus once contiguous panmictic population suddenly converts to smaller isolated subpopulations (Corlatti et al. 2009). Direct mortality from

vehicle collisions is the most immediate threat to population persistence (Puky 2006). Amphibian movement in particular is a multi phase process consisting of juvenile dispersal and adult migration (Pittman et al. 2014) which makes them susceptible to higher road mortality. To access spatially separated patches such as breeding site, foraging site and dispersal phase they need

Roads fragmenting wildlife habitat create impacts listed in Fig 2.



to cross roads in fragmented landscapes (Andrews et al. 2008). Their vulnerability is further increased by their relatively slow movement rates (Hels & Buchwald 2001), and many species become immobile in response to approaching vehicles (Mazerolle et al. 2005).

The highest rates of road mortality for amphibians occur where roads are located in the vicinity of a wetland or pond disrupting the spatial connectivity of essential resources and habitats across the landscape (Ashley & Robinson 1996; Dodd & Smith 2003). The studies of amphibian mortality due to roads are highest surveyed in Europe (Puky 2006; Puky 2003; Schmidt & Zumbach 2008). Mass movements triggered by rainfall and warm weather may result in excessive rates of road mortality for salamanders and anurans (Turner 1955; Clevenger et al. 2001)

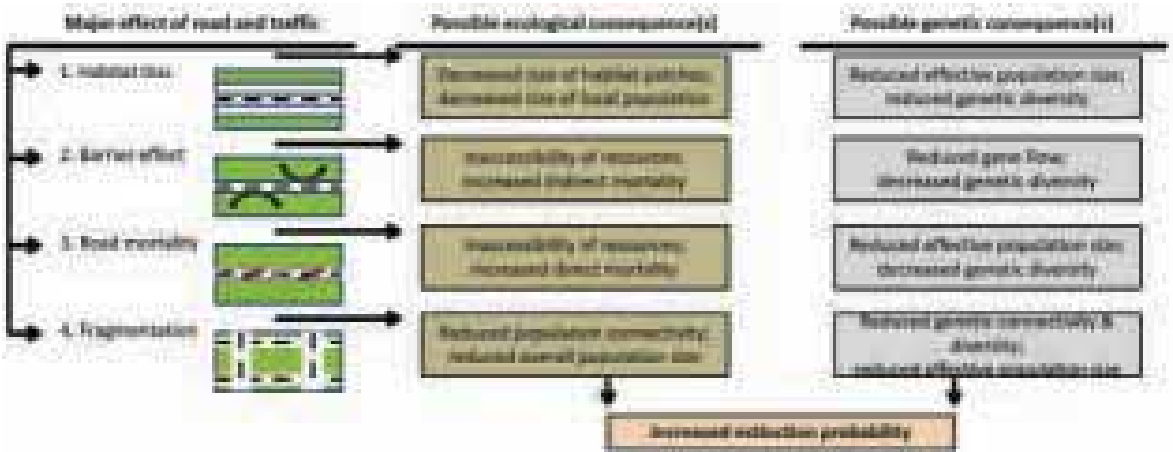
Amphibian and reptile species often have restricted or patchy distributions and small effective population sizes. Little is known about the long-lasting ecological effects of roads on animal populations in terms of reduced mobility, increased isolation and/or splitting of gene pool. Roads may serve as barriers that restrict gene flow and decrease genetic diversity through a combination of direct mortality and inbreeding. In



Fig 4: *Duttaphrynus melanostictus* killed while in amplexus

functionally-small populations, these effects may significantly increase inbreeding depression and increase the probability of local extinction (Rodriguez et al. 1996). Few studies have empirically documented genetic effects on herpetofauna due to roads, and few support the hypothesis that roads reduce gene flow and decrease genetic diversity in amphibians (Reh & Seitz 1990; Hitchings & Beebee 1998; Lesbarrès et al. 2003; Balkenhol & Waits 2009). A study investigated the land use and roads on the genetic structure of common frog (*Rana temporaria*) and found that separation by highways reduced the average amount of heterozygosity (genetic variation) in the population (Reh & Seiz 1990).

Fig 5: Flowchart explaining road impacts (left), their ecological (centre) and genetic (right) consequences. Adapted from Fahrig (2002) and Jaeger (2014). Modified from Balkenhol & Waits (2009).



Road ecology in Indian perspective

Road ecology is a hot topic in conservation biology. Enormous amount of literature deals with road related mortality on various taxa ranging from macro invertebrate soil fauna (Haskell 2000), herpetofauna (van

Gelder 1973; Cooke 1995; Rosen & Lowe 1994; Fahrig et al. 1995; Haxton, 2000; Gibbs & Shriver 2002; von Seckendorff Hoff & Marlow 2002; Andrews & Gibbons 2005, Glista et al. 2008, Elzanowski et al. 2009 and Langen et al. 2009), birds (Mumme et al. 2000) and mammals (Laurance et al.



Fig 6. Amphibian road kills are often not detected on road.

2006). Scanty information is available in this regard in Indian context.

Gokula (1997), Vijayakumar et al. (2001), Sundar (2004), Das et al. (2007), Kannan (2007) and Rao & Girish (2007); Baskaran & Boominathan (2010) have studied the effect of vehicular traffic on animal mortality, although the studies are seasonal and for very short duration.

Only handful of studies in India show evidence that amphibians are more susceptible to vehicular traffic (Vijayakumar et al. 2001; Sundar 2004; Bhupathy et al. 2011; Seshadri & Ganesh 2011; Sharma 2015). However, mortality of animals also depends on other factors such as time of the year, habitat and/or landscape characteristics, behavior of the species and road and traffic characteristics. Andrews and Gibbons (2005) also noticed that presence of breeding habitats near to roads lead to higher amphibian mortality.

In the Anamalai hills of southern India, Western Ghats, Vijaykumar et al. (2001) sampled highway segments passing through rainforest fragments and tea gardens. Their study found 72 reptile and 311 amphibian (5 families and 3 species) road kills including several endemic species. It is interesting to note that the study recorded greater mortality among amphibians (2 individuals/km and 6 individuals/ 10 km) than reptiles (0.43 individuals/Km) due to vehicular movement. In case of reptiles, more than 80% (N=49) of the road kills were that of snakes and in case of amphibians, the family Bufonidae represented half (46.6%, N=144) the total

number of individuals recorded. As per the study, an increased activity of amphibians and uropeltids was observed during rainfall, thus making them more vulnerable to road traffic. Conservative extrapolation would suggest that a 100 km stretch of road through forests here witnesses an annual kill of around 10,000 amphibians and reptiles, a large proportion accounting for endemic species to the Western Ghats.

A short study for three months recorded 68 instances of road kill reptiles belonging to 21 species from near Kaziranga National Park in Assam (Das et al 2007). However, similar report from many protected areas fragmented by roads is hitherto missing.

Sheshadri et al. (2009) carried out a survey of amphibian mortality on roads in Sharavathi river basin in the central Western Ghats. Road kill data was recorded in three different land use areas: agricultural fields, water bodies and forests for four days along three 100m stretches in each type of area. A total of 144 individuals belonging to 2 orders, 8 families, 11 genera and 13 species were recorded in the survey. Kills/km was observed for an overall average of 40 kills/km and road kill species compositions varied significantly between land use areas.

A road mortality study of herpetofauna along National Highway 220 in Western Ghats has recorded mortalities of 101 amphibians and 78 reptiles in 24 days. The study showed significant relationship of mortality of amphibians with time of the day and influence of climate on amphibian activity. Overall 3.5 amphibians/ 10 km and

2.7 reptiles/ 10 km were recorded dead during the study. (Bhupathy et al. 2011).
Mortality of herpetofauna has been reported from Western Ghats by Boominathan (1999) in Mudumalai (19 amphibians/ 10 km; 8.3 reptiles/ 10 km) between December 1998 to March 1999 & Mukherjee (2007) in Anaikatti hills (3.49 reptiles/ 10 km) between January 2003 to December 2004. Several other studies by Selvan et al. (2012) and Nagar et al. (2013) show impacts of vehicular traffic

Table 1: Review of Studies on impact of roads and vehicular traffic on Indian Amphibian populations.

S. No	Family	Species	Total mortality	Season of Study
1	Bufonidae	<i>Duttaphrynus melanostictus</i>	145	May- June 1988
	Ranidae	-	73	
	Rhacophoridae	-	35	
	Ureotyphilidae	<i>Ureotyphlus</i> sp.	19	
	Ichthyophidae	<i>Ichthyophis</i> sp.	8	
	Unidentified	-	31	
2	Bufonidae	<i>Duttaphrynus melanostictus</i>	17	June 2008
	Dicroglossidae	<i>Euphlyctis cyanophlyctis</i>	3	
	Dicroglossidae	<i>Fejervarya</i> cf. <i>rufescens</i>	1	
	Dicroglossidae	<i>Fejervarya</i> sp.	22	
	Dicroglossidae	<i>Hoplobatrachus tigerinus</i>	1	
	Microhylidae	<i>Microhyla ornata</i>	9	
	Microhylidae	<i>Uperodon</i> cf. <i>montanus</i>	1	
	Nyctibatrachidae	<i>Nyctibatrachus</i> sp.	3	
	Ranidae	<i>Hylarana malabarica</i>	1	
	Ranixalidae	<i>Indirana</i> sp.	2	
	Rhacophoridae	<i>Polypedates</i> cf. <i>occidentalis</i>	1	
	Ichthyophiidae	<i>Ichthyophis</i> sp.	4	
	Ichthyophiidae	<i>Ichthyophis beddomei</i>	5	
	Unidentified anurans		74	
3	Bufonidae	<i>Duttaphrynus melanostictus</i>	42	December 2006- November 2007
	Ranidae	<i>Indosylvirana temporalis</i>	4	
	Ranixalidae	<i>Indirana</i> sp.	48	
	Rhacophoridae	<i>Polypedates pseudocruciger</i>	1	
	Rhacophoridae	<i>Rhacophorus malabaricus</i>	3	
	Dicroglossidae	<i>Sphaerotheca breviceps</i>	3	
4	Amphibians	-	211	2008- 2009
5	Amphibians	-	125	January 2007 - June 2007
6	Bufonidae	<i>Duttaphrynus melanostictus</i>	11	November 2015
	Dicroglossidae	<i>Hoplobatrachus</i> sp.	1	
	Dicroglossidae	<i>Sphaerotheca</i> sp.	1	
	Microhylidae	<i>Uperodon</i> sp.	1	
	Rhacophoridae	<i>Polypedatus</i> sp	2	
	Unidentified anurans		247	

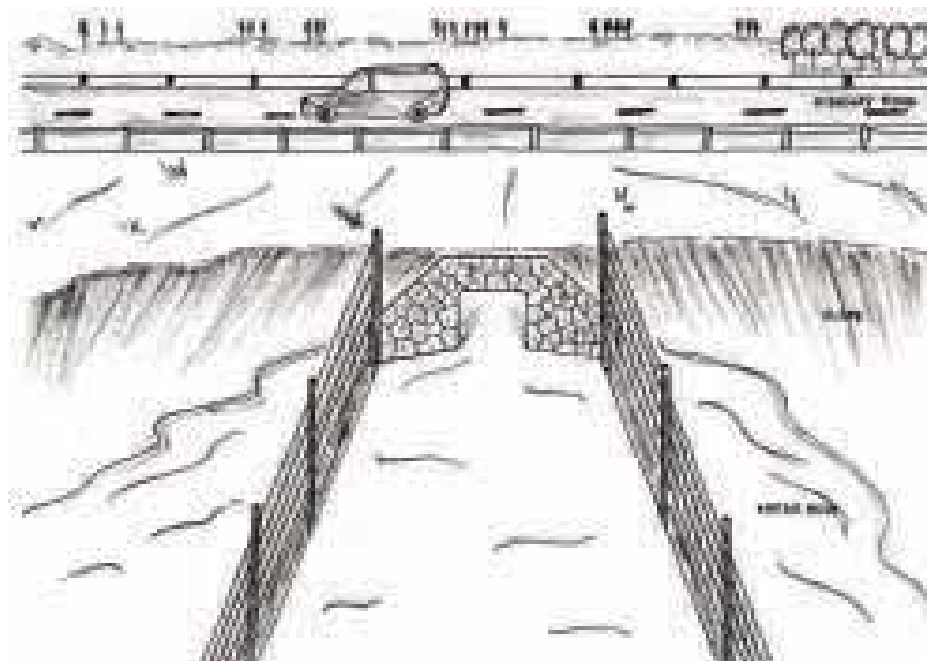


Fig 7: A fenced waterway to guide amphibians through the underpass. Adapted from Gilbert et al. (2015). Illustration by Preeti Sharma.

Mitigation Strategies

Mitigation refers to 'measures to avoid any harm' or 'lessen the gravity of an offence'. Mitigation strategies refers to 'step wise approach to reduce harm', to habitat and wildlife. In present context of development of linear infrastructures such as roads, railway lines, and transmission lines, the adverse impacts on wildlife are well documented (Jalkotzy et al. 1997; Seiler 2002; van der Ree 2015). While the impacts caused due to such linear infrastructures cause direct mortality (Forman et al. 2003) but roads also act as a source of noise, light and chemical pollution, thus creating barriers to amphibian movement (Bee & Swanson 2007; Dorsey et al. 2015).

This brings in the need for wildlife crossing structures popularly known as 'green infrastructure' (Forman et al. 2003) that can be deployed for safe passage of amphibians across the landscape. Role of crossing structures is to provide safe movement for an animal to cross the road and offer connectivity between habitats along the road. Some of the methods mentioned below may be applied to minimize and prevent impacts of roads and check road mortality of lesser vertebrates or group such as amphibians.

The ecological effects of roads get manifested in the regional and local

populations and in turn, affect individual behavior. It is, therefore important that while designing mitigation strategies SMART (refers to measures that are specific, measurable, achievable, realistic and timely) approach is recommended for greater effectiveness (Forman et al. 2003).

One of the greatest factors influencing the risk of road mortality in amphibians is their movement or dispersal patterns, species-specific life history traits and behavior (Forman et al. 2003; Andrews & Gibbons 2005). An animal moves either to disperse to new sites or settle near natal sites and often need to cross fragmented landscapes by roads and railway lines in order to select spatially separated breeding and foraging sites (Andrews et al. 2008). The relatively slow movement rate (Hels & Buchwald 2001) and seasonal amphibian movement (juvenile dispersal and adult migration) makes amphibians more susceptible to road mortalities (Pittman et al. 2014). These factors have strong consequences for individual fitness, gene flow, natural selection, adaptation, population persistence, metapopulation dynamics and species distribution (Knowlton & Graham, 2010).

Despite the importance of movement to the persistence of species, there are considerable gaps in our understanding of movement processes (Bonte et al. 2012;

Clobert et al. 2009; Ronce 2007). Thus, mitigation measures to reduce mortality due to vehicles involve a combination of approaches: erecting fences and walls to exclude wild animals from moving towards the road and prevent road mortality, constructing of overpasses and underpasses to allow safe movement across the road, erecting signage and warning systems as a regulatory measure to alter human behavior. By incorporating taxa specific designs and choosing the right locations for crossing structures, can keep mortality of wildlife on roads at bay.

Mitigation specific to amphibian population

The road-crossing structures can be installed beneath the road surface (<1.5 m diameter or height) or above the road as fencing or deflectors along the road to direct the movement of amphibians. Potential measures for mitigation of amphibian mortality on roads are listed briefly:

- A) **Amphibian tunnels:** Tunnels installed between breeding habitats, or between isolated wetlands known to allow for migration of adult amphibians and emigration of metamorphs from breeding ponds (Podlucky 1989). A tunnel may be designed with a funneling device to the entrance to direct flow of animals entering the tunnel. The closer the tunnels are to the breeding habitats, the more accepted by the amphibians and serve as springboards for amphibians crossing roads (Eriksson et al. 2000).
- B) **Pipe Culverts:** These are dry passages, typically round in shape designed to facilitate overland movement of amphibians, made up of smooth metal, or concrete. Single or multiple pipes may be placed at appropriate position and facing the direction of movement of amphibians. Plantation of native vegetation along the sides of the culverts improves the efficacy of the culvert and provides refuge to the amphibians.
- C) **Wildlife Culverts:** These are passages up to 120 cm wide located over water channels and are designed for movement of smaller or lesser

vertebrates. They may serve as potential amphibian crossing passage provided they are located at the right places.

- D) **Modified Culverts/ Drainage Culverts:** These are water passage and drainage which can be modified for wildlife passage. Although the potential of these structures as successful wildlife passage had been overlooked (Forman et al. 2003), yet these non-wildlife-engineered structures can prove best for linkages of species populations (Rodriguez et al. 1996).
- E) **Fences:** These are structures designed over the surface of road to prevent movement across roads instead get directed towards wildlife passages. Fences have been practically efficient and effective measure for amphibian crossing. If used in combination with the right crossing structures placed at the right spot, the animals are successfully directed to the tunnels or culverts placed along the fence. Wire, chain link, rail, plastic mesh or concrete can be used for constructing of fencing for amphibians.

Prerequisites for amphibian mitigation

An assessment of species composition (threatened species if any), relative abundance and habitat association along the road stretch is a must in order to design species-specific mitigation measure. Some of the methods to identify zones of animal activity are visual encounter survey, road survey, radiotelemetry, mark recapture using Visual Implant Elastomer (VIE) or Passive Integrated Transponder (PIT) tags (Heyer et al. 1994).

- A. Before implementing any linear infrastructure project, it is important to map availability of amphibian breeding (streams, ponds, lakes etc) habitats along proposed road side in a GIS domain. It is better if alignment can be made at least 1km away from amphibian breeding site. With limited data on amphibian movement in Indian context it is difficult to exactly define a distance class, of our native species

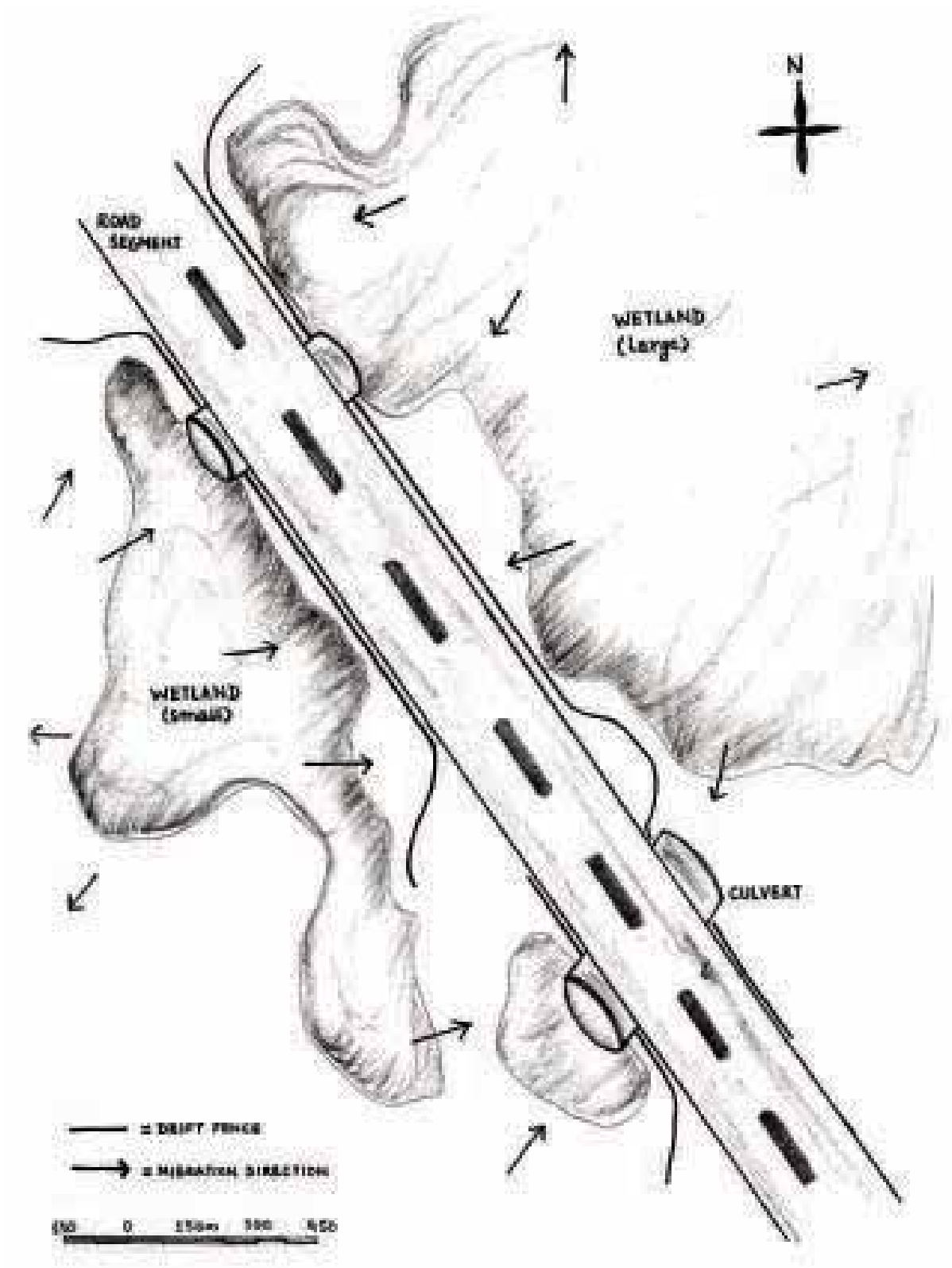


Fig 8: Schematic representation of the use of culverts coupled with fencing to provide safe passage for amphibians. Adapted from Aresco (2005). Illustration by Preeti Sharma

during a particular dispersal/migratory event.

- B. If roads bisect amphibian habitat and migration routes, specially designed underpasses need to be established. Such small or large passage if placed across peak activity zone, these structures can significantly help reduce the fragmentation effect. Traditional culverts and minor bridges (built on ditches, streams and rivers) can be modified to facilitate safe movement of amphibians under the highway.
- C. The position and size (length and diameter) of these structures, and the angle that they are approached by amphibians are critical factors determining the success of these specialized structures. Amphibians often prefer square shaped tunnels that are buried close to the ground surface, rather than those that are round and set deep into embankments.
- D. Passageways should be designed in a way to minimize environmental gradients. Amphibians are particularly sensitive to moisture condition and thus cement flooring of passage may not provide ideal condition for amphibian movement. Cement flooring also likely to limit natural vegetation growth may prove unsuitable for cryptic animals. There should not be gaps or breakage in the fencing. The material should be durable and it should be made sure that the passage ways connects the two sides of the habitat properly.
- E. Lack of light within tunnels may deter usage by amphibians (Jackson & Tynning, 1989, Krikowski, 1989). Thus, larger tunnels (such as those in NH) are required to be designed in a way to provide sufficient light to facilitate passage use (Puky 2003). This can be achieved by maximizing diameter to length ratio of passageways to maximize light penetration and airflow.
- F. Tunnels fitted with metal grates on the top (on road surface) may act as effective "small passage for amphibians. Metal grates at the top of passageways may be installed to facilitate light and air penetration.
- Gaps on the metal grates will help amphibians to drop down in the passage.
- G. In dry areas, engineering solution for maintaining moisture in passageways needs to be done. In case underpass falls on a waterway or wetland, provision to fence the side of the water channel need to be envisaged.
- H. Fencing is necessary where amphibians need to be channeled to use a particular crossing facility. Most fences act as one-way barriers so that the movement of amphibians is only prohibited in a single direction. Top of the fences should curve away from the highway, in order to prevent tree frogs to climb over fences (Puky 2003). Fences should be at least 50 cm high; however, in case of fencing for both reptiles and amphibians they need to be at least 0.8 m high above ground and 0.2 m below ground. In order to direct animals towards passageways to facilitate use, fencing in "Zig-Zag fashion (Jackson & Tynning 1989), is most useful.
- I. Traffic noises are known to have auditory masking effect amphibians (Parris et al 2009). If road is passing through forested areas (especially in western Ghats or Northeast India) mitigation measures should include construction of noise barrier of the synthetic fiber of height 1.5m and same may be constructed on the rigid crash barrier on either side of the highway as long as forested corridor section of road.
- J. Amphibian translocation as possible mitigation measure is still a controversial topic. However, in certain cases where entire breeding sites or land areas are to be lost and cannot practicably be replaced for technical reasons, amphibian population may be considered for short distance 'in situ' transfers, as a likely mitigation measure. Expert intervention is necessary in this aspect. The new site should be close to the original breeding site and should have same hydrological and ecological conditions as far as possible.

- K. Construction of new breeding sites or restoration of earlier breeding site of amphibians may also help maintain native amphibian population and thus can be visualized as possible mitigation measure. Pond designs should take into account measures to assist in their long term management for amphibians. Studies may be undertaken to determine the number, size and type of breeding sites that will be needed to provide effective mitigation. Pond design may vary according to biological requirement of targeted species or amphibian assemblages. If new ponds are close to the road, care should be taken to separate their catchment from the road drainage system, to avoid pollution.
- L. Amphibian breeding site management may include the removal of extensive growth of aquatic vegetation or removal of weeds from surrounding areas of the wetland. Care should be taken to remove predatory fish from such breeding habitats.

Follow up

Installation of amphibian fencing, tunnels and underpasses mark the beginning of a mitigation project. Passageways and fencing systems require monitoring for repairs and maintenance. Intensive monitoring and a follow up research may be needed in order to demonstrate effectiveness of passageways and fencing as effective mitigation measure for amphibian road kills. There should be strong research component associated with mitigation measures and funds for that need to be assured. Costs of maintenance and specialist monitoring, should also be included in the provisions for long-term management.

Conclusion

India with an annual economic growth of ca. 7.5 percent for the fiscal year 2016-2017 (Ministry of Finance 2016), the pace of infrastructure development is inevitably set to increase in forth coming years. As is the need of the hour, new contracts have been awarded for 5331 km length of National highways while 3480 km have been already constructed in 2015-16 (Ministry of Road

Transport and Highways). The Vehicular densities on Indian roads, that have increased from 0.3 to 30 million in the past 50 years (Raman 2009; Seshadri et al. 2009) are going to go further up in coming days.

As conservationists, we cannot stop progress but we surely can shape it (Rosner 2013). Plenty of studies that support the avoidance of roads by larger mammals like elephants, rhinoceros, lions and tigers. However, scanty knowledge is available for Indian amphibians. Suitable mitigation strategies can be formulated and proposed for implementation if we have scientific evidence that show detrimental effects of roads on Indian amphibians and reptiles. This could help in designing the right mitigation plan at species specific level. Nevertheless, efforts should be made to construct more passages or alter existing structures in the future to lower habitat fragmentation along transportation infrastructure.

Scientific studies surely are important to back up any mitigation plan, but it is also up to the managers to look out for effective conservation strategies to balance the situation. It is high time that we feel the need to have a strategic landscape-level planning in India (Saxena et al. 2016) and practice Strategic environment assessment for developmental projects at landscape level. It is important to incorporate the conservation proponents in the decision making process in order to achieve conservation objectives in Indian transportation system.

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Fig 9. Non-engineered structures can be well utilized for road kill mitigation measures.

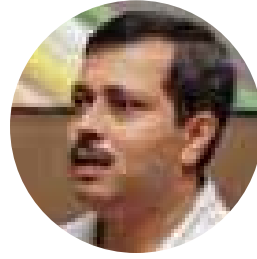


**ABOUT THE
AUTHORS**



Prof. Sushil K. Dutta

Prof. Dutta is currently working as a visiting scientist at National Centre for Biological Sciences, TIFR Bangalore. A veteran herpetologist contributed extensively to the current knowledge on amphibians of India and Sri Lanka. His research interest includes systematics, evolution and developmental Biology in reptiles and amphibians. He is the man behind Department of Science and Technology-Govt. of India Sponsored School in Herpetology that trained more than hundred researchers in professional herpetology.



Dr. Karthikeyan Vasudevan

Dr. Vasudevan works as a Principal Scientist at Laboratory for Endangered Species Conservation, Center for Cellular and Molecular Biology, Hyderabad. His research interest includes evolutionary biogeography and systematics with a focus on Asian amphibians and reptiles. He has described several novel species to science and published extensively on poorly known ecological aspects of endemic and threatened herpetofauna.



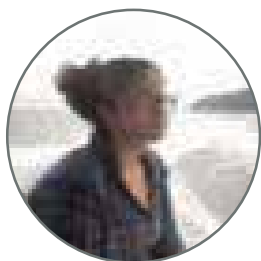
Arun Kanagavel

Arun Kanagavel is a research associate at the Conservation Research Group, Kochi and a founding trustee of the Women in Conservation and Climate Action Network. His research interests are focused towards improving the existing knowledge of lesser known amphibians, understanding their social associations and in multi-stakeholder initiatives that involve their actual conservation on the ground.



Dr. Lilly Margaret Eluvathingal

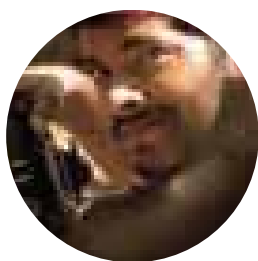
Dr. Lilly Margaret Eluvathingal is currently an adjunct professor at Occidental College, USA. She worked for several years studying communities of anurans occupying tea plantations in Kerala, India. She also volunteers at the Los Angeles Natural History Museum where she is learning the basics of museum curation. She is interested in ecology education, and inspiring young adults into pursuing careers in Ecology and Wildlife Conservation.

**Sethu Parvathy**

Sethu Parvathy is a research associate at the Conservation Research Group(Kochi) and the President and founding trustee of the Women in Conservation and Climate Action Network. Her research interest is focused towards community-based biodiversity conservation and its association with forest-dwelling communities and wildlife. This is beyond consumptive or sustenance purposes and includes tradition and culture. Understanding the knowledge of such communities could relate to a key source of information about species ecology and population trends and involving them could contribute to holistic management of biodiversity.

**Prof. (Mrs.) R.N.K. Hooroo**

Prof. (Mrs.) R.N.K. Hooroo, Developmental Biology Laboratory, North Eastern Hill University, Shillong and her research group have been working in the study of amphibians with special reference to Meghalaya, North East India. Survey and documentation of amphibian fauna in some select regions of Meghalaya and studies on the breeding biology and development of anurans prevalent in the State has been carried out. Further, analysis on the effects of biotic factors, physico-chemical factors and some pesticides on the breeding, morphology and development of anurans are being carried out.

**Sandeep Das**

Sandeep Das is research scholar at the Kerala Forest Research Institute, Peechi. He is interested in ecology and behavior of amphibians and has been working on amphibians of Kerala since 2010. He is currently also an EDGE fellow working on the endangered Purple Frog.

**Dr. Ramachandran Kotharambathis**

Dr. Ramachandran Kotharambath is an Assistant Professor at the Department of Animal Science, Central University of Kerala, Kasargod. His research interest is primarily in the biology of caecilians - subterranean limbless amphibians. He has been working on their taxonomy, systematics and molecular phylogeny in the southern Western Ghats and has also discovered two new species of caecilians.

**Dr. Pratyush P. Mohapatra**

Dr. P Mohapatra is working as a scientist in Zoological Survey of India at Central Zone Regional Centre, Madhya Pradesh. He is working on diversity, distribution and conservation of herpetofaunal species in Eastern ghats and Central India. His research interest lies in resolving taxonomic status of various herpetofauna in India. Beside herpetology, he contributed significantly towards inventory of Birds, butterflies, scorpions and plants in the Eastern Ghat landscape.

**Preeti Sharma**

Preeti is currently working as a Research Biologist at UNESCO Category 2 Centre based at the Wildlife Institute of India, Dehradun. She is looking at the diversity and distribution of endemic amphibians of Sahyadri Tiger Reserve, Maharashtra to formulate a long term monitoring method for such lesser known and cryptic species. Her primary research interests focuses on impact of linear infrastructures (roads, railways, powerlines) on wildlife. She believes that among vertebrate's amphibians and reptiles are most effected from linear infrastructure projects in India and a lot to be done to mitigate such massacre. Her hobbies are herping, trekking, painting and writing.

**Dr. Basundhara Chettri**

Dr. Chettri's research interest lies in the field of ecology, conservation biology, herpetology and Environment Impact Related works. Current research focuses on spatial distribution pattern and conservation of herpetofauna (amphibians and reptiles) in the Eastern Himalaya. She is also interested to look into the impact of climate change on the faunal distribution of the Himalayas. Apart from hard core research, she is also developing interest to document the indigenous knowledge of the ethnic communities.

**Brij Kishore Gupta**

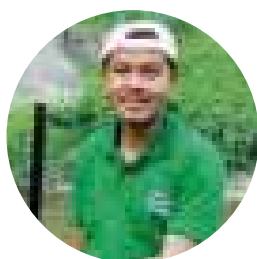
Dr Gupta's research interest include Ex situ conservation of Amphibians, Conservation Planned breeding of Amphibians, behaviour and Biology of Amphibians, Enrichment of Animals Habitats in Captivity for their welfare and well being and Conservation Education

**Dr. Cuckoo Mahapatra**

A developmental biologist by profession, Dr. Mahapatra is working as Assistant Professor in P. G. Department of Zoology at North Orissa University. Apart from teaching and guiding students she is currently working on biology and regeneration of various amphibian species of Odisha.

**S. Harikrishnan**

Mr. Harikrishnan is keenly interested in ecology and conservation biology of reptiles and amphibians. For his PhD thesis, he studied the assembly and structure of lizard communities in the Andaman & Nicobar Islands. He described new species of Lizards and amphibians and published research article that addresses herpetological research methodology.

**Dr. Uttam Saikia**

Dr. Saikia is currently working as a scientist in Zoological Survey of India, Shillong. His primary research interest involves small mammalian systematic s especially bats and rodents. He has field experience in the western and Eastern Himalaya of India and is presently engaged in detailed documentation of the bat fauna of northeastern India.

**Vishal Kumar Prasad**

Vishal interested in the field of systematics, ecology and bioacoustics with prime focus on herpatofauna. He also like to study, compare and evaluate ecosystems and identifying their roles in conservation of biodiversity with special focus on species endemism, richness and diversity within various biogeographic regions such as Western Ghats, Avarallis and Central Indian Landscape. He has a Master's degree in Environmental Science and Technology from BharatiVidyapeeth Institute of Environmental Education and Research (Pune) where he studied distribution of Herpetofauna in Biodiversity Hotspecks in Western Ghats. Moreover, he has worked on vocalization and call pattern some anurans.



Prof. Indraneil Das

Prof. Neil is Professor at the Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak. His research and teaching interests include zoological systematics, herpetology, vertebrate biology, history of natural history and biogeography.



Prof. (Mrs) Pravati K. Mahapatra

Prof. Mahapatra teaches in the PG Department of Zoology, Utkal University, Bhubaneswar, India. Research interest include regeneration studies in anuran tadpoles; lysosomal enzyme mediated cell death and haematology of anuran tadpoles; skeletochronology of herpetofauna.



Prof. S.N. Bahuguna

Prof. S.N. Bahuguna, Ph.D., D.F.Sc. (Poland) is Professor in Department of Zoology and Biotechnology, H.N.B. Garhwal (Central) University, Srinagar Garhwal, Uttarakhand and has 35 years of teaching and research experience. He has published more than 80 national and international research papers in the field of Ichthyology, Batrachology, Mammalogy, and Animal Tissue Culture. He has completed 10 research projects funded by ICAR, UCOST, DBT, GBPIHED, and UGC. He has guided and supervised 25 scholars for D.Phil. and Ph.D. Degree. He attended and chaired number of conferences, workshop and presented scientific papers at National and International level.



Bitupan Baruah

Born in Assam and received his MSc. degree in Wildlife & Biodiversity Conservation from North Orissa University, Odisha. His special interest is on natural history, systematics and distribution of Amphibians and Snakes. He has also a number of publications on odonates and butterflies. He received small grant from Forest Dept. of Odisha Govt. to carry out work on Herpetofauna of Hadgarh Wildlife Sanctuary, Odisha in 2015. Joined in Wildlife Institute of India, Dehradun in 2016 and worked as Project Biologist in "X-mas Bush Frog Conservation Project, Meghalaya. Currently he is working as Project Fellow in WII-NMCG Project.

**Dr. Ashish Kumar Chowdhary**

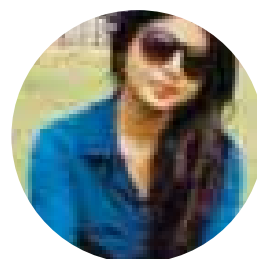
Dr. A. K. Chowdhary, is research scholar in Department of Zoology and Biotechnology, H.N.B. Garhwal (Central) University, Srinagar Garhwal, Uttarakhand and has around 6 years of teaching and research experience. He has published 12 national and international research papers and 3 book chapters in the field of Ichthyology, Batrachology, and Animal Tissue Culture. He was awarded Basic Scientific Research Fellowship in Sciences for meritorious students funded by UGC. He has an experience of working in two research projects funded by DBT and NMHS-GBPIHED. He has been an awardee of Young Scientist Awards by UGC and UCOST. His research interest includes Taxonomy, Cytogenetics, Molecular Taxonomy, Ecology, and Conservation Biology of Fish and Amphibians.

**Prof. Mithra Dey**

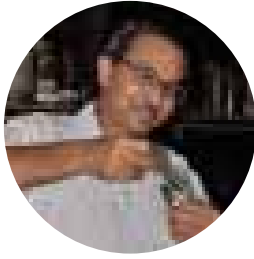
Mithra Dey, Ph.D., is Professor in the Department of Ecology and Environmental Science, Assam (Central) University, Silchar, Assam, India. She is actively involved both in teaching and research. Her field of interest is Freshwater ecology and Ecotoxicology with special interest in Herpetofauna, diversity and ecology of anurans, impact of pesticides and heavy metals on anurans and fish. Her research work also includes studies on Solid Waste Management. She has published more than 30 papers in national and international journals and presented many papers in national and international conferences. Seven scholars have been awarded Ph. D under her supervision till date.

**Sunkam Narayana Ramanujam**

Sunkam Narayana Ramanujam completed Ph.D. in 1982 and is working in the Department of Zoology, North Eastern Hill University, Shillong, Meghalaya, India, for past 12 years. Currently he is the Professor and Head of the Department of Zoology. His main interest of research includes eco-physiology, fish biology and environmental toxicity. He has published more than 40 papers in national and international journals and presented many papers in national and international conferences. He has supervised eight Ph.D. students successfully.

**Pammi Singh**

Pammi Singh was Research Scholar and Project Fellow (UGC) in the Department of Ecology and Environmental Science, Assam (Central) University, Silchar, Assam, India. She has been awarded PhD degree under this university in July 2017. She also received M.Sc. degree in Ecology and Environmental Science from Assam University, Silchar, in 2010. Her work focuses on study of habitat ecology, feeding ecology and heavy metal accumulation in anuran tadpoles from different types of habitats.

**Dr. Kaushik Deuti**

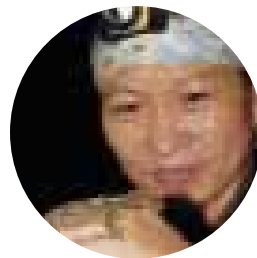
Dr. Deuti works as a Scientist at Zoological Survey of India, Kolkata. Dr. Deuti is an amphibian taxonomist. For his PhD he studied Ecology and Biology of *Chiromentissimus* and since then actively involved in amphibian research. He described several new species of frogs and also published field guides on Salamanders, Lizards and birds. His hobby include bird watching, photography and creative writing.

**Prof. Saibal Sengupta**

Prof. Sengupta works as a professor at Don Bosco University, Guwahati. One of the pioneer in amphibian studies of Northeast India. Dr. Sengupta described several new species to science and reported faunal inventory report for several protected areas of Assam. His interest include biological studies and amphibian taxonomy with special reference to ranid frogs of the genus *Amolops*.

**Dr. Jayanta Gogoi**

Dr. Gogoi is an Assistant Professor in Zoology at Arya Vidyapeeth College, Guwahati. For his PhD he worked on Ecology, Biology and Taxonomy of Rhacophorid frogs of the genus *Polypedates*. His work revealed that detail observation of natural history information may provide clue towards cryptic species recognition. His interest include teaching, capacity building and photography.

**Dr. Rachunliu G. Kamei**

Dr. Kamei did her PhD on Caecilians with special reference to Northeast India. Her interest include Amphibian systematics and Biogeography. She is a recipient of prestigious Marie Curie Fellowship for her research on Caecilian Systematics.



Dr. H T Lalremsanga

Dr. H T Lalremsanga works as an Assistant Professor at Department of Zoology, Mizoram University. His research interest include herpetology systematics and he has described new species of snakes and fishes.



Dr. Ronald K. L. Tron

Assitant Professor
Department of Zoology
St. Edmund's College Shillong



Dr. Duwaki Rangad

Assitant Professor
Department of Zoology
St. Edmund's College Shillong



Dr. Shanwell Khongwir

Assitant Professor
Department of Zoology
Shillong College, Shillong



Miss. Caressa M Thabah

Research Scholar
Department of Zoology
NEHU, Shillong



Dr. (Mrs.) Asorphi Janai Iangrai

Assitant Professor
Department of Zoology
Synod College, Shillong



भारतीय वन्यजीव संस्थान
Wildlife Institute of India



Wildlife Institute of India

Chandrabani, Dehradun-248001, India,

Tel.: +91 135 2640111-115, Fax.: +91 135 2640117

Email: wii@envvis.nic.in; envvis@wii.gov.in,

Website: <http://wiienvvis.nic.in>; <http://wii.gov.in/envvis>